



NUtraceutical TRreatment for hYpercholesterolemia in HIV-infected patients: The NU-TRY(HIV) randomized cross-over trial



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HIGHLIGHTS

- HIV infection and antiretroviral therapy (ART) are associated with a complex disturbance of lipid metabolism.
- Mild low-density lipoprotein cholesterol (LDL-C) elevations may be successfully controlled by the nutraceutical combination.
- The nutraceutical combination reduced PCSK9 levels, subclinical inflammation and aortic stiffness.

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ABSTRACT

Background and aims: Despite hypercholesterolemia has been recognized to increase cardiovascular risk in human immunodeficiency virus (HIV)-infected patients, cholesterol-lowering therapy is underused in this population, due to fear of drug-drug interactions with antiretroviral therapy (ART).

We investigated the effects of a nutraceutical combination (NC) on lipid profile, proprotein convertase subtilisin/kexin type 9 (PCSK9), subclinical inflammation and arterial stiffness in ART-treated HIV-infected patients. **Methods:** This was a prospective randomized open-label trial with a cross-over design including 30 stable HIV-infected patients on ART with low-density lipoprotein cholesterol (LDL-C) > 115 mg/dL, not taking lipid-lowering treatment. After a 3-week lipid stabilization period, the effects associated with 3 months of an oral NC containing red yeast rice and berberine vs. no active treatment (noNC) were assessed for plasma total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), lipoprotein(a), PCSK9, high-sensitivity C-reactive protein (hs-CRP) levels and aortic pulse wave velocity (aPWV).

Results: At baseline, significant correlations between PCSK9 levels, age ($\rho = -0.51, p = 0.004$), waist circumference ($\rho = 0.36, p = 0.005$) and CD4⁺ cell count ($\rho = -0.40, p = 0.027$) were observed. NC treatment effects corrected for noNC were significant for TC ($-14\%, p < 0.001$), LDL-C ($-19\%, p < 0.001$), PCSK9 ($-12\%, p = 0.02$), hs-CRP ($-14\%, p = 0.03$) and aPWV ($-6\%, p = 0.005$). No significant effects were observed for HDL-C, TG and lipoprotein(a). NC treatment was safe and no significant alterations in muscle, liver and immunovirological parameters were observed. No carry over effect was recorded.

Conclusions: The tested NC significantly reduced plasma cholesterol and PCSK9 levels, attenuated subclinical inflammation and improved arterial stiffness in stable HIV-infected patients on ART.

1. Introduction

Human immunodeficiency virus (HIV)-infected patients taking

antiretroviral therapy (ART) are at increased cardiovascular (CV) risk [1]. Beyond traditional CV risk factor exposure, HIV infection *per se*, concomitant immunovirological abnormalities and ART have been

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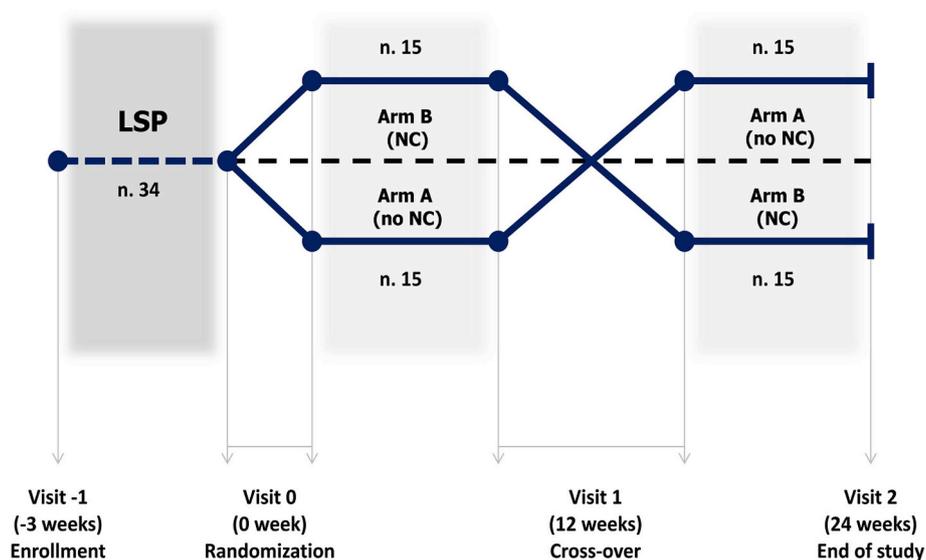


Fig. 1. Scheme of the study protocol.

LSP, lipid stabilization period; NC, nutraceutical combination; noNC, no active treatment.

shown to contribute to increased CV disease burden in this population [2]. Specifically, exposure to different ART regimens has been associated with proatherogenic lipid abnormalities [3], including hypercholesterolemia, hypertriglyceridemia and hypoalbuminemia [4]. Moreover, despite extensive ART-mediated virological suppression, residual HIV infection may still sustain chronic low-grade inflammation [5]. Noteworthy, both dyslipidemia and systemic inflammation are strongly associated with surrogate indices of preclinical atherosclerosis, including arterial stiffness, intima-media thickness and endothelial dysfunction [6–8], predicting an increased CV risk in the HIV population [6–9].

The efficacy of statin therapy in reducing plasma low-density lipoprotein cholesterol (LDL-C) levels and inflammation in HIV-infected patients has been consistently demonstrated [10]. In addition, statins have been found to be effective in improving different surrogate markers of CV disease in this population [11,12]. However, although around 80% of HIV-infected patients have an abnormal lipid profile, statin use is reported to be less than 10% in these patients [13]. Fear of drug-drug interactions and subsequent adverse events are believed as the main justifications for this low prescription rate [14].

In the general population, a nutraceutical-based approach, in addition to lifestyle changes, has been proposed as an integrative cholesterol-lowering strategy before statin initiation [15]. This approach is supported by accumulating evidence on the beneficial effects of various phytochemicals on lipid profiles and their favourable tolerability when used at their lower doses [16]. Moreover, different nutraceutical combinations (NCs) claiming lipid-lowering activity have been found to exert also some anti-inflammatory effects [17,18]. Several interventional studies have reported that an NC of red yeast rice-derived monacolin K, berberine, policosanol, astaxanthin, folic acid and coenzyme Q10 can significantly reduce plasma total cholesterol (TC), LDL-C and high-sensitivity C-reactive protein (hs-CRP) levels [19,20]. Moreover, the same NC has been shown to improve arterial stiffness and endothelial function and to reduce endothelial injury in patients with low-moderate hypercholesterolemia [19,21].

To date, a number of studies have investigated the lipid- and inflammation-lowering effects of various nutraceutical compounds, including monacolin K, in HIV-infected patients [22–25]. However, neither the efficacy and safety of berberine, nor the combined effects of an NC containing low-dose monacolin K and berberine on lipid profile, low-grade inflammation and subclinical vascular damage have ever been assessed in an HIV-infected population.

In this cross-over interventional study with a blinded end-point evaluation, we investigated the effects of an NC containing red yeast rice-derived monacolin K, berberine, policosanol, astaxanthin, folic acid and coenzyme Q10 on serum lipid profile, lipoprotein(a), proprotein convertase subtilisin/kexin type 9 (PCSK9) and hs-CRP levels, as well as arterial stiffness in stable HIV-infected patients on ART.

2. Patients and methods

2.1. Study subjects

Between April 1st 2016 and April 30th 2017, 34 HIV-infected patients were consecutively recruited for this cross-over interventional study from the Day Hospital of the Infectious Diseases Clinic in Perugia. Stable patients on ART, defined as those with persistent HIV-RNA levels below 20 copies/mL and CD4⁺ cell counts above 400/μL for at least 6 months, with LDL-C > 115 mg/dL and free of CV disease were enrolled. Exclusion criteria were: age under 18 years, diabetes, current pregnancy, estimated Glomerular Filtration Rate (GFR) < 60 mL/min (calculated using the “Modification of Diet in Renal Disease-4” – MDRD-4 - equation), liver impairment [aspartate transaminase (AST) and/or alanine transaminase (ALT) > 3 times upper limit of normal], opportunistic infections over the past three months, having received an organ transplant or recent interferon therapy, and current or recent (≤ 6 months) treatment with lipid-lowering drugs. Data regarding demographic issues, medical history, comorbidities, immunovirological profile and current medications were collected. The level of physical activity was estimated using the Borg Rating of Perceived Exertion Scale at baseline and at the end of the study. The study was approved by the local Ethics Committee of the University of Perugia (Perugia, Italy) and the trial was registered in *ClinicalTrials.gov* PRS (NCT03470376; Unique Protocol ID: 2015-006). The methods were performed in accordance with the relevant ethical guidelines and regulations. Informed consent was obtained from all patients prior to enrolment.

After enrolment (Visit – 1), all patients were prescribed a 3-week lipid stabilization period with a standardized diet regimen (Fig. 1). Phone interviews were carried out once a week in order to ask about adherence to the prescribed diet regimen. In addition, all the patients were instructed to maintain their habitual pattern of physical activity and not alter their prescribed ART over the study period. At the end of the lipid stabilization period, 4 patients refused to continue the study because they were not able to guarantee treatment adherence. Visits

were scheduled for 30 patients at baseline (Visit 0), 12 weeks (Visit 1; cross-over) and 24 weeks (Visit 2; end of study). At Visit 0, patients were randomly assigned to a 1:1 ratio to receive as active treatment (Arm B) a 3-month once-daily oral therapy with a patented proprietary NC containing red yeast rice extract 200 mg (equivalent to 3 mg monacolin), policosanol 10 mg, berberine 500 mg, astaxanthin 0.5 mg, folic acid 0.2 mg and coenzyme Q10 2 mg (Armolidip Plus[®], MEDA Pharma - Mylan) or to receive a 3-month no active treatment (noNC) (Arm A). Moreover, all of the enrolled patients were advised to continue the prescribed standardized diet regimen.

We planned a 2-arm cross-over study, with restricted randomization (random permuted blocks and phone central randomization), with LDL-C (primary outcome measure) as a continuous response variable. Random allocation sequence, enrolment of participants and assignment of treatment were done by different physicians. Patients were not blinded to treatment assignment, but were to outcome evaluation. At Visit 1, the cross-over between treatment arms occurred: patients who were allocated to Arm A were assigned to Arm B and *vice versa*. At Visit 2, compliance to therapy, treatment effect and safety were evaluated. The primary outcome with respect to treatment effect was the change in the LDL-C baseline level after 3 months. The secondary outcomes with respect to treatment effect were changes in PCSK9 and hs-CRP baseline levels and arterial stiffness after 3 months. Additional analyses were performed for other pre-specified outcome measures concerning safety: immunovirological parameters and indices of both muscle damage and hepatic function.

2.2. Clinical evaluation, laboratory parameters and assessment of arterial stiffness

Clinical evaluation, and the assessments of laboratory parameters and arterial stiffness were performed at baseline (Visit 0), at 12 weeks (Visit 1) and at 24 weeks (Visit 2). All of these exams were performed at our medical center at 8.00 a.m. The examination rooms were maintained between 21 and 23 °C. All of the exams followed a 13-h overnight fast. Height and weight were measured to the nearest 0.1 cm and 0.1 Kg, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters and waist circumference (WC) was measured. Brachial blood pressure was measured with a mercury sphygmomanometer after patients had sat for 10 min or longer [26]. On average, 3 measurements were taken for this analysis. Regarding the blood workup, TC, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and glucose were measured by adopting an enzymatic colorimetric method (Autoanalyzer KONE-PRO; DASIT S.p.A, Cornaredo, Milano, Italy), LDL-C was calculated using the Friedewald equation, PCSK9 was measured via a sandwich-ELISA method (Elabscience Biotechnology Co. Ltd, Wuhan, China), lipoprotein(a) was measured with a latex turbidimetry assay (Giese Diagnostics s.r.l., Rome, Italy), hs-CRP levels were assessed using nephelometry (BN100; Siemens Dade Behring, Siemens S.p.A., Milan, Italy), CD4⁺ cell count was determined by flow-cytometry analysis (Citomics FC 500; Beckman Coulter, Brea, USA) through whole blood staining with anti-CD45-PC5 and anti-CD4-PE fluorescent antibodies (Beckman Coulter, Marseille, France), and HIV-RNA levels were measured using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0 (Roche Molecular Systems, NJ, USA). Blood samples were also tested for AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and creatine phosphokinase (CPK) (AU5800 Clinical Chemistry System, Beckman Coulter s.r.l., Cassina De' Pecchi - Milano, Italy). Aortic pulse wave velocity (aPWV) was assessed non invasively using an automatic device, the SphygmoCor Vx system (AtCor, Sydney, Australia), as previously described [27–29]. This exam uses a single-lead ECG and a high-fidelity applanation tonometer to measure the pressure pulse waveform sequentially in two peripheral artery sites, one at the base of the neck for the common carotid artery and the other over the femoral artery. Transit time of the pressure pulse waveform was calculated using the R-

wave on the ECG as reference. A graduated calliper was used to calculate the path length, that is, the distance between the carotid artery site of measurement and the sternal notch subtracted from the distance between the femoral artery site and the sternal notch. Therein, aPWV was calculated using the formula $aPWV \text{ (m/s)} = \text{distance (m)} / \text{transit time (s)}$ [27–29]. All of these measurements were performed by the same physician. The intra-observer reproducibility of aPWV was 5.0%. For our study, the ten-year risk of fatal cardiovascular disease was estimated by using the SCORE-European low-risk chart [30]. The proportion of patients reaching the recommended LDL-C goals was calculated based on current European and American guidelines for the treatment of dyslipidemias [30,31].

2.3. Statistical analysis

SPSS statistical package, release 17.0 (SPSS Inc, Chicago, Ill) was used for the statistical analyses. Values are expressed as the mean \pm standard deviation (SD). Base 10 logarithmic (LG) transformation was performed for skewed variables and the LG-values were used. Paired samples *t*-test and Wilcoxon rank-sum test were used to compare within group differences of the investigated variables at baseline and after 3 months of treatment. Independent sample *t*-test and Mann-Whitney *U* test were used to compare changes in the variables between the treatment orders (AB vs. BA). Carry over was assessed by comparing the sum of the variable responses (response/1 + response/2) between the treatment orders (AB vs. BA). Correlation analyses were performed using the Pearson's and Spearman's coefficients of correlations. It was calculated that a 25-patient sample size was needed for this two-arm cross-over study, in order to reach a 80% probability of detecting a treatment difference at a two-sided 0.05 significance level, whenever a difference in plasma LDL-C between treatments was 25 mg/dL. This was based on the assumption that the within-patient SD of LDL-C was 30 mg/dL. The SD of baseline LDL-C and the expected LDL-C reduction were hypothesized in light of the results from a previous intervention study [21] as well as a recent meta-analysis using the same NC [20]; in these studies, LDL-C SD and NC-mediated LDL-C reduction were 29 mg/dL and 24 mg/dL, respectively.

3. Results

The characteristics of the study population are described in Table 1. 67% of the enrolled patients were male, 50% were overweight or obese, 27% were hypertensives, and 53% were current smokers. In addition, 67% of the patients had hypertriglyceridemia (TG > 150 mg/dL), 20% hypoalphalipoproteinemia (HDL-C < 40 mg/dL) and all of the enrolled patients had LDL-C > 115 mg/dL (an inclusion criteria). The entire enrolled population had an optimal baseline immunovirological control (CD4⁺ cell count > 400/mL, HIV-RNA < 20 copies/mL) and it remained constant for the entire duration of the study. Additionally, in the entire population, the distribution of the ART regimen was the following: protease inhibitors 53%, nucleoside reverse transcription inhibitors 83%, non-nucleoside reverse transcription inhibitors 47% and integrase inhibitors 17%. Regarding the use of concomitant therapies, 5 patients (17%) were under an anti-hypertensive treatment (4 patients were on ACE inhibitors and 1 was taking a β -blocker) and 1 patient had been prescribed a benzodiazepine plus a serotonin reuptake inhibitor.

3.1. Baseline correlations

At baseline, plasma PCSK9 levels were associated with age ($\rho = -0.51, p = 0.004$), WC ($\rho = 0.36, p = 0.0048$), and CD4⁺ cell count ($\rho = -0.40, p = 0.027$). An association between aPWV with WC ($\rho = 0.39, p = 0.032$) and systolic blood pressure ($\rho = 0.52, p = 0.003$) was found. Plasma lipoprotein(a) levels were associated with TC ($\rho = 0.40, p = 0.027$), LDL-C ($\rho = 0.44, p = 0.014$) and HDL-C

Table 1
Baseline characteristics of the study participants.

	Total (n = 30)
Age (years)	44 ± 10
Gender (% men)	67
Caucasian race (%)	93
Current smoking (%)	53
Physical activity (score)	10.6 ± 3.3
Body mass index (kg/m ²)	25.3 ± 3.1
Waist circumference (cm)	90.7 ± 9.1
Systolic blood pressure (mmHg)	128 ± 13
Diastolic blood pressure (mmHg)	76 ± 9
Total cholesterol (mg/dL)	245 ± 30
LDL cholesterol (mg/dL)	162 ± 23
HDL cholesterol (mg/L)	47 ± 10
Triglycerides (mg/dL)	180 (124–222)
Lipoprotein(a) (mg/dL)	13.3 (7.1–20.9)
PCSK9 (ng/mL)	214 (117–381)
hs-CRP (mg/L)	1.7 (1.2–2.9)
Aortic PWV (m/sec)	7.3 ± 1.7
CD4 ⁺ cell count (n/μL)	774 ± 271
CD4 ⁺ cell count > 400/μL (%)	100
HIV-RNA level < 20 copies/mL (%)	100
eGFR (mL/min/1.73 ²)	95 ± 25

LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; hs-CRP, high sensitivity C-reactive protein; PWV, pulse wave velocity; eGFR, estimated glomerular filtration rate.

levels ($\rho = 0.44, p = 0.014$). WC was associated with TC ($\rho = 0.44, p = 0.015$), LDL-C ($\rho = 0.38, p = 0.038$), and TG levels ($\rho = 0.37, p = 0.042$). No evidence of an impact from ART and/or concomitant therapies was observed at baseline levels of the studied variables.

3.2. Treatment effect

Treatment effect (NC vs noNC) was significant for the following variables (Table 2): TC (−14%, $p < 0.001$), LDL-C (−19%, $p < 0.001$), PCSK9 (−12%, $p = 0.02$), hs-CRP levels (−14%, $p = 0.02$) and aPWV (−6%, $p = 0.005$), whereas no significant impact from NC vs. noNC was observed on HDL-C, TG and lipoprotein(a) levels. In addition, BMI, WC, blood pressure and glucose levels were not significantly affected by

Table 2
Treatment effect of the nutraceutical combination (NC), weighted for the effect of noNC.

	Change ^a , SD	p
Body mass index (kg/m ²)	−0.2 ± 1.6	0.39
Waist circumference (cm)	−0.8 ± 2.7	0.13
Systolic blood pressure (mmHg)	−1.9 ± 7.9	0.19
Diastolic blood pressure (mmHg)	−1.2 ± 5.3	0.22
Total cholesterol (mg/dL)	−33 ± 19	< 0.001
LDL cholesterol (mg/dL)	−30 ± 14	< 0.001
HDL cholesterol (mg/L)	0.6 ± 6.2	0.56
Triglycerides (mg/dL)	−19 ± 71	0.15
Glucose (mg/mL)	−1.1 ± 3.4	0.10
Lipoprotein(a) (mg/dL)	−2.4 ± 17.8	0.46
PCSK9 (ng/mL)	−30 ± 67	0.02
hs-CRP (mg/L)	−0.3 ± 0.7	0.02
Aortic PWV (m/sec)	−0.4 ± 0.7	0.005

Independent samples *t*-test and Mann-Whitney *U* test were used to compare changes in the study variables between treatment orders.

LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; hs-CRP, high sensitivity C-reactive protein; PWV, pulse wave velocity.

^a Change is calculated as: post-treatment value in the NC - baseline value in the NC; post-treatment value in the noNC - baseline value in the noNC.

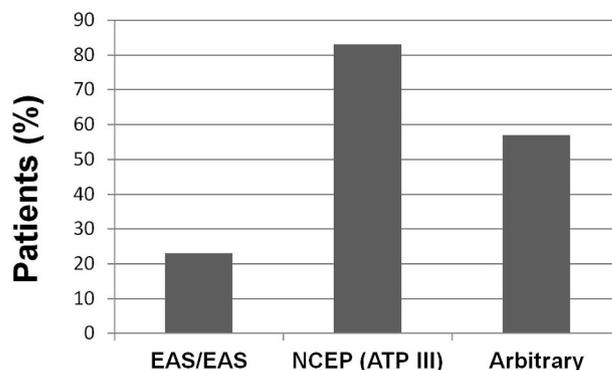


Fig. 2. Proportion of patients reaching specific LDL-C goals. The “EAS/ESC” LDL-C goal is < 115 mg/dL for both low and moderate risk patients; the “NCEP (ATP III)” LDL-C goals are < 160 mg/dL and < 130 mg/dL for low and moderate risk patients, respectively; the “Arbitrary” LDL-C goals have been proposed as < 130 mg/dL and < 115 mg/dL for low and moderate risk patients, respectively. EAS, European Atherosclerosis Society; ESC, European Society of Cardiology; NCEP (ATP III), National Cholesterol Education Program (Adult Treatment Panel III).

either NC supplementation or noNC treatment. No significant associations were observed between baseline TC and LDL-C and their treatment-induced changes. Moreover, ART, concomitant therapies and level of physical activity did not significantly influence any of the observed treatment-induced changes in the studied variables. No evidence of carry over and period effects were recorded for any of the studied variables.

The proportion of patients reaching the recommended LDL-C goals are represented in Fig. 2.

3.3. Safety

Plasma AST, ALT, GGT, ALP and CPK levels were not significantly affected by either NC supplementation or noNC intervention (Fig. 3). In addition, GFR remained stable in both treatment arms (noNC: 96 ± 27 mL/min, NC: 98 ± 27 mL/min, $p = NS$), as well as CD4⁺ cell count (noNC: 803 ± 289 n/μL, NC: 781 ± 316 n/μL, $p = NS$) and the number of patients with HIV-RNA copies < 20 copies/mL (100% for both treatment arms).

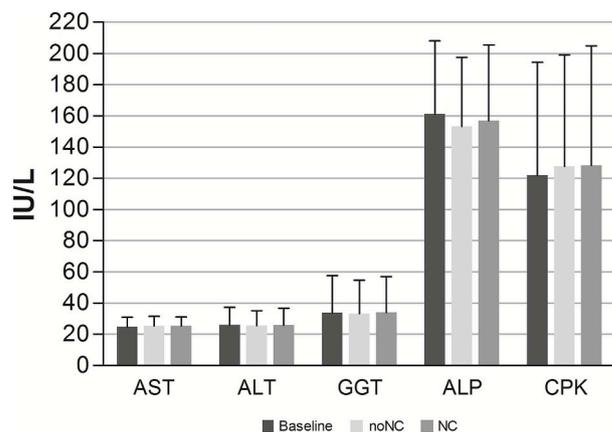


Fig. 3. Safety of the nutraceutical combination (NC) versus noNC, as determined by measurement of liver and muscle function parameters. Black bars represent baseline values, grey bars post-treatment values in the NC and noNC arms. AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; CPK, creatine phosphokinase.

4. Discussion

This is the first interventional study assessing the effects of an oral NC containing red yeast rice-derived monacolin K, berberine, policosanol, asthaxanthin, folic acid and coenzyme Q10 on serum lipids, PCSK9 and hs-CRP, and arterial stiffness in stable HIV-infected patients on ART and without diagnosed CV comorbidities.

The NC supplementation resulted in a significant cholesterol-lowering effect. To the best of our knowledge, only one previous randomized double-blind placebo-controlled study has reported a significant reduction in both TC and LDL-C plasma levels (-31 mg/dL and -32 mg/dL, respectively) after a short-term oral supplementation with only red yeast rice (monacolin K 5 mg) in ART-treated HIV patients [23]. In our study, the magnitude of the NC-mediated TC and LDL-C reductions (-33 mg/dL and -30 mg/dL, respectively) supports the results of this past study along with other investigations, including trials and meta-analyses, exploring the lipid-lowering effects of this NC in non HIV-infected patients [19,20,23,32]. These reductions were associated with the achievement of the recommended goals for plasma LDL-C levels in a number of patients; however, it is still debated which risk calculator and LDL-C treatment goals should be used for HIV-infected patients on ART [33–35]. Although the cholesterol-lowering impact from each component of this NC cannot be drawn from this research, it may be assumed that monacolin K and berberine exerted the greatest effect, according to previous data showing their significant cholesterol-lowering activities. To this regard, it has been shown that monacolin K is able to inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, whereas berberine can upregulate the hepatic LDL receptor and inhibit PCSK9 expression [15].

From a clinical perspective, the results of this study may have an impact on the approach to treat mild hypercholesterolemia in stable HIV-infected patients on ART without diagnosed CV comorbidities. In fact, as unfavourable drug-drug interactions have been reported between statins and ART, a low-dose NC along with lifestyle modifications could be considered as a safe approach for these patients before prescribing statins.

In our study, no safety concerns emerged from the use of low-dose NC. In particular, liver, muscle, renal and immunovirological parameters were not affected by the 3-month supplementation with this NC. However, it should be emphasized that monacolin K has the same chemical structure as lovastatin. Noteworthy, the pharmacokinetic properties of lovastatin are similar to simvastatin and they both undergo cytochrome P450 (CYP) 3A4 metabolism [36]. Both simvastatin and lovastatin are contraindicated in HIV patients receiving ART [36], due to expected interactions with some antiretroviral drugs which may increase serum statin levels (*i.e.*, protease inhibitors and integrase strand transfer inhibitors) [37]. Thus, in our study, the lack of clinical and laboratory adverse effects in the patients receiving a lovastatin-like NC could be attributed to the very low dose of monacolin K.

Regarding the observed effect of this NC on PCSK9 levels, we found these levels to be significantly reduced. Despite not having been able to demonstrate the mechanisms of this effect, it is plausible that it might be associated with the known ability of berberine to downregulate PCSK9 expression [38]. Whether the observed plasma PCSK9 reduction may have some clinical relevance remains to be elucidated. On the one hand, the observed PCSK9 level reduction was mild in our study, which might not be clinically relevant. Conversely, plasma PCSK9 levels, which show a trend toward an increase in HIV-infected patients compared to healthy controls, are directly associated with higher cholesterol levels and CV disease risk [39,40].

Another result emerging from our study was a modest but significant NC-mediated hs-CRP reduction, which might suggest a mild beneficial effect of this NC on low-grade systemic inflammation, as previous studies have already reported [18,19]. Therefore, it is plausible that the NC-mediated cholesterol-lowering effect may have mediated the attenuation of the systemic low-grade inflammation in

this population. In fact, there is evidence that cholesterol-lowering may attenuate chronic low-grade inflammation mostly by promoting liver X receptors activation and counteracting toll-like receptor signalling and inflammasome activation [41]. However, a direct NC-mediated anti-inflammatory action, independent of cholesterol reduction, might have contributed to this effect. In fact, some anti-inflammatory effects of astaxanthin and coenzyme Q10 have been reported, though not always consistently [42–44]. Irrespective of the mechanism leading to our observed hs-CRP reduction, it is worth mentioning that a significant CV benefit can be anticipated from the combined LDL-C and hs-CRP reduction in the general population [45]. Moreover, we have previously seen that the reduction of both plasma LDL-C and hs-CRP levels in patients with primary hypercholesterolemia was associated with a reduction in arterial stiffness, an indirect marker of atherosclerosis and CV risk [46]. However, whether this result might translate into a possible CV benefit in stable ART-treated HIV-infected patients needs to be explored.

In our study, the NC supplementation resulted in a significant attenuation of arterial stiffness. Previous studies have shown that this NC improved several indices of preclinical vascular damage, suggesting its anti-atherogenic effect beyond lipid-lowering [19,21]. As discussed above, we hypothesize that the NC-mediated effect on both LDL-C levels and low-grade systemic inflammation may have contributed to the observed reduction of arterial stiffness. Accordingly, both hypercholesterolemia and systemic inflammation are known to promote arterial stiffening [46–48]. Conversely, cholesterol reduction and attenuation of low-grade systemic inflammation have been reported to improve aPWV in hypercholesterolemic patients [46–48]. To this regard, it has been suggested that cholesterol-lowering and attenuation of inflammation are able to promote positive changes in the elastic elements of the arterial walls and also improve endothelial function [49]. However, to determine whether this NC-mediated improvement in aortic stiffness along with LDL-C and hs-CRP reductions may benefit CV clinical endpoints, larger studies are needed.

In the present study, a non-significant 19 mg/dL triglyceride reduction was found. In a recent large and more statistically powered meta-analysis of 14 RCTs using the same NC in non HIV-infected patients [20], a significant 14 mg/dL triglyceride reduction was reported. However, irrespective of the statistical significance of the triglyceride reduction in this meta-analysis, that was most likely driven by the statistical power, the extent of this reduction was modest. Other therapeutic strategies need to be considered to better treat HIV-related hypertriglyceridemia, which may reach a prevalence of 56% in stable HIV-infected patients on ART [50]. For example, the switching of an ART regimen to a more lipid-friendly one, maintaining virologic suppression, adhering to lifestyle changes or prescribing pharmacological therapy (*e.g.*, omega-3 fatty acids, fibrates, tesamorelin) may be valid therapeutic options [51].

In addition to the non-significant NC-mediated impact on triglycerides, we were also unable to find a significant effect of this NC on plasma HDL-C levels. Concerning this issue, some previous studies in non HIV-infected patients have reported a significant HDL-C level increase, while other studies have not [52]. Whether the specificity of the HIV-infected population on ART, compared to other HIV-free populations, might explain our results, is an issue that needs further investigation in properly designed prospective case-control intervention trials. However, we found borderline associations between the hsCRP plus triglyceride changes and the HDL-C change ($\rho = -0.33$, $p = 0.07$ and $\rho = -0.36$, $p = 0.05$, respectively). It is reliable that statistical significance was not reached for these associations probably due to the small population size of the study. Nonetheless, the extent and direction of these correlations suggest that a putative and indirect HDL-C raising effect of this NC is possible, when a more intense triglyceride-lowering and attenuation of the systemic inflammation are reached. Finally, the above discussed findings cannot exclude a possible impact from this NC on HDL functioning. To this regard, HDL

dysfunction has been observed in HIV-infected patients [53] and it can be fostered by several HIV-associated abnormalities, including hypertriglyceridemia and systemic inflammation [54]. In addition, it has been suggested that the pharmacological activation of AMP-activated protein kinase (AMPK) may enhance HDL function and anti-atherogenic properties *in vivo* (e.g., reverse cholesterol transport, anti-inflammatory, anti-oxidative) [55]. Since berberine is a recognized AMPK activator [56], it could be hypothesized that a NC containing berberine might contribute to improve HDL function. However, an assessment of HDL function has not been performed in our study; hence, this hypothesis remains speculative.

In our study, at baseline, plasma PCSK9 levels were associated positively with WC and negatively with CD4⁺ cell count. A variable association between PCSK9 levels and different indices of adiposity in the HIV-negative population has been previously reported. In line with our findings, previous studies have shown either a plasma PCSK9 level increase in overweight and obese patients compared to normal weight individuals or a positive association between PCSK9 levels and measures of adipose tissue accumulation [57,58]. In contrast, a negative correlation between plasma PCSK9 levels and measures of central obesity has been reported in young females [59]. Also, individuals with a genetically determined PCSK9 deficiency, due to loss-of-function R46L variant, had higher BMIs and increased fat masses compared with controls [60]. Finally, no association between plasma PCSK9 levels and measures of fat accumulation has been reported in the general population and in HIV-infected patients [39,61]. Overall, several factors may explain such a divergence of results, including gender, age, genetic background, HIV infection, type of ART and lack of measuring PCSK9 activity [39,60,62].

Conflicting results from past studies have been reported concerning a possible association between PCSK9 levels and CD4⁺ cell count in HIV-infected patients [39,63]. Specifically, PCSK9 levels were negatively associated with CD4⁺ cell count in HIV-untreated patients, whereas this association was lost in HIV-infected patients receiving protease inhibitors [39]. Also, a PCSK9 polymorphism (i.e., *rs17111557*) has been associated with CD4⁺ levels in HIV/hepatitis C virus co-infected women [50]. Hence, despite the paucity of literature on this issue, it might be hypothesized that the negative association between PCSK9 and CD4⁺ levels might be the result of either a causative or a reverse association, where PCSK9 might promote HIV infectivity (low CD4⁺ cell count) or HIV infection might increase PCSK9 levels.

Hence, the relationship between PCSK9 levels, on one hand, and adipose tissue accumulation and CD4⁺ cell count on the other hand, needs to be further investigated along with the mechanisms underlying these associations.

Some limitations of this study should be taken into account. First, its small population size may have compromised the reliability of the results. However, all the assumptions for statistical power calculations were satisfied in this study, thus rendering our results robust. Second, the treatment effects were recorded over a short-term period. Third, a wash-out period was not planned for this cross-over study, which might have possibly interfered with the final results. However, a 3-month treatment period is considered long enough to abrogate the cholesterol-lowering efficacy of the most potent statins. No evidence of carry over effect was recorded for all the studied variables. Fourth, the non-use of a food frequency questionnaire and a PCSK9 activity measurement needs to be acknowledged.

In conclusion, the present study provides supporting evidence that an NC containing low-dose monacolin K and berberine might have beneficial effects on lipid profile, systemic inflammation and subclinical atherosclerosis in stable HIV-infected patients on ART with mild hypercholesterolemia and without diagnosed CV comorbidities. Large and well-designed randomized clinical trials are warranted to further test the long-term efficacy and safety of this approach in primary prevention for CV disease in this population.

Clinical trial number

ClinicalTrials.gov PRS (NCT03470376; Unique Protocol ID: 2015-006).

Conflicts of interest

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

Author contributions

Conception and design of the study: Matteo Pirro, Daniela Francisci, Amirhossein Sahebkar, Franco Baldelli. Acquisition of data: Vanessa Bianconi, Elisabetta Schiaroli, Massimo R. Mannarino, Francesco Barsotti, Andrea Spinuzzi, Francesco Bagaglia. Analysis and interpretation of data: Matteo Pirro, Daniela Francisci, Massimo R. Mannarino, Amirhossein Sahebkar, Franco Baldelli. Drafting the article or revising it critically for important intellectual content: Matteo Pirro, Daniela Francisci, Vanessa Bianconi, Elisabetta Schiaroli, Amirhossein Sahebkar, Franco Baldelli. All authors have approved the final article.

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