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Paraoxonase-1 activities in individuals with different HDL circulating levels: Implication in reverse cholesterol transport and early vascular damage

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HIGHLIGHTS

- HDL-associated PON1 could represent one of the determinants of the biological functions of this lipoprotein.
- High/low HDL-C levels are not accompanied by proportional PON1 activity changes.
- PON1 specific activity is lower in hyperalphalipoproteinemia patients than in Controls.
- The combination PON1/Apo A-1 is inversely related to subclinical atherosclerosis.

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ABSTRACT

Background and aims: Epidemiological data showing that high-density lipoprotein cholesterol (HDL-C) is inversely associated with cardiovascular disease have led to the idea that cholesterol contained in this lipoprotein may be protective. Against, recent evidence suggests that the athero-protection from HDLs may result from other functions, unrelated to the carried cholesterol. HDL accessory proteins, such as paraoxonase 1 (PON1), have been suggested to endows HDL with antioxidant and anti-inflammatory properties and to contribute to the athero-protective function of the lipoprotein. We aimed to evaluate whether extreme fluctuation in HDL-C levels correlates with PON1 activity.

Methods: Levels of PON1-related arylesterase and lactonase were assessed in subjects with primary hyperalphalipoproteinemia (HAL, HDL-C > 90th percentile), hypoalphalipoproteinemia (HA, HDL-C < 10th percentile) and controls. Cholesterol efflux capacity (CEC) through several pathways and other metabolic parameters and markers of vascular disease were also determined.

Results: Despite the marked change in HDL-C and Apoliprotein A1 (APO A1) ($p < 0.001$ for all comparisons), arylesterase and lactonase were only slightly increased in HAL compared with HA subjects ($p < 0.05$), but not *vs.* controls. This change in PON1 activities was no longer significant after adjustment for either HDL-C or APO A1. Both enzymatic activities were positively associated only with aqueous diffusion CEC ($r = 0.318$, $p < 0.05$ and $r = 0.355$, $p < 0.05$, respectively) and negatively with the presence of plaques ($p < 0.05$).

Conclusions: We showed that extreme high/low HDL-C levels are not associated with equal increase/decrease in PON1 activities. This enzyme appears to contribute to the HDL role in reverse cholesterol transport and anti-atherosclerosis processes. Further investigation is required to corroborate our findings.

1. Introduction

Epidemiological studies consistently support the concept of high-density lipoprotein cholesterol (HDL-C) as a strong inverse predictor of cardiovascular risk [1,2]. However, genetic polymorphisms influencing HDL-C levels do not consistently associate with CVD risk [3]; moreover,

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interventional studies with HDL-C raising therapies generated inconsistent clinical outcomes [4,5]. Collectively, these findings suggest that the sole cholesterol content of HDL particles does not fully capture the HDL-related atheroprotective functions. Indeed, the functionality of HDL also stem from their capacity to exert anti-inflammatory and antioxidant activities. This well-recognized pleiotropic nature of HDL mostly relates to its major non-lipid constituents, i.e. apoliprotein A1 (Apo A1), apo A-2, apo E, and other accessory proteins, *in primis* Paraoxonase 1 (PON1) [6–11].

Paraoxonase-1 (PON1) is an esterase/lactonase enzyme almost exclusively associated with circulating HDL [6]. Despite intense efforts in understanding PON1 role, its catalytic mechanism and physiological significance are still a matter of debate. Endogen lipophilic lactones, such as those resulting from fatty acid oxidation (e.g. 5,6-dihydroxytrienoic acid 1,5-lactone) or from the pathway of homocysteine catabolism (e.g. acyl-homoserine lactones), are the most likely physiological substrates of PON1 catalysis [12,13]. Through this activity, PON1 appears to contribute in protecting HDL, low density lipoprotein (LDL), macrophages, and endothelium from oxidative and inflammatory challenges, and to stimulate cholesterol efflux from macrophages [6,11,14,15]. PON1 can also exert arylesterase and paraoxonase activities on synthetic chemicals [12,16], the former being the most measured activity in epidemiological studies; indeed, it is minimally influenced by some prevalent PON1 genetic polymorphisms, thus disclosing low inter-individual variability [16–18]. The putative role of PON1 in promoting HDL biological functions might account for the epidemiological evidence linking low arylesterase or lactonase activity with high cardiovascular and neurodegenerative disease risk [19–23].

Hyperalphalipoproteinemia (HAL) is a heterogeneous syndrome characterized by very-high HDL-C (over the 90th percentile of the general population); it is caused by different diseases, organ malfunctions and genetic factors. It is not clear whether subjects bearing this condition also disclose low CV-risk [24,25]. In a recent report, it has been shown that HAL patients have sub-clinical vascular damage and cholesterol efflux capacity comparable to normolipemic controls [26]. To test the hypothesis that in HAL the absence of putative CVD risk reduction may be at least partially ascribed to inadequate improvement in PON1 function, we compared arylesterase and lactonase plasma activity in three groups of individuals: a) subjects with primary HAL; b) subjects with hypoalphalipoproteinemia (HA: HDL-C < 10th percentile); c) subjects with normal levels of HDL-C (controls). In addition, taking into account the paucity of human studies on PON1-capacity to promote cholesterol efflux from macrophages, we explored the possible association between PON1 activities, HDL-promoted cholesterol efflux capacity (CEC), and sub-clinical atherosclerotic indexes.

2. Patients and methods

2.1. Subjects

The study was conducted on three groups of individuals:

1. HAL group: twenty subjects (18 females and 2 males; mean age: 52 years) with HDL-C ≥85 mg/dL for women and ≥75 mg/dL for men (90th percentile of the local reference population), in at least two consecutive serum samples. The previously reported genetic characterization of HAL subjects highlighted a high prevalence of a common polymorphism on *CETP* gene (SNP p.Val422Ile) [26];
2. HA group: twenty subjects (11 females and 9 males; mean age: 49 years) with HDL-C < 35 mg/dL for men and women (10th percentile of the local reference population) in at least two consecutive serum samples.
3. Control group: twenty subjects (18 females and 2 males; mean age: 51 years) with HDL-C within normal range (> 35 mg/dL AND < 75 mg/dL in men OR < 85 mg/dL in women).

Participants were enrolled among subjects attending the metabolic outpatient clinic of Sant'Anna University Hospital (Ferrara, Italy). All were in good health and none had liver or renal function test abnormalities. Hypothyroidism, pregnancy, alcohol consumption > 10 g daily, active treatment with hormones or lipid-modifying drugs were considered exclusion criteria. Other details on inclusion/exclusion criteria have been reported previously [26].

Patients and controls were clinically evaluated by interview and physical examination; blood pressure, and anthropometric parameters were also measured. This study conforms to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was conducted accordingly to Good Clinical Practice guidelines. It was approved by the Local Ethics Committee; written informed consent was obtained from each patient and no personal information was available to Authors (blinding).

2.2. Serum sampling and biochemical assays

Venous blood samples from patients were collected after overnight fasting and serum stored at −80 °C until analyzed.

Total and unesterified cholesterol (TC and UC), HDL-C, triglycerides, and glucose were assayed by standard enzymatic-colorimetric methods; LDL-C was calculated according to the Friedewald formula. Apo A1 and Apo B were determined by immunoturbidimetry.

Both arylesterase and lactonase assays were performed by UV-VIS spectrophotometric assays in a 96-well plate format by using a Tecan Infinite M200 microplate reader (Tecan group Ltd, Switzerland).

Arylesterase activity was assessed by using 1 mmol/L phenylacetate, in 9 mmol/L Tris-HCl (0.9 mmol/L $CaCl_2$, pH 8) [22]. A molar extinction coefficient of $1.3 \times 10^3\ L^{-1}\ mol^{-1}\ cm^{-1}$ was used for calculation of enzymatic activity, expressed in kilo unit per liter. One unit of arylesterase activity accounts for 1 μmol of phenol produced in a minute under the conditions of the assay. Intra-assay CV was 3.8% whereas inter-assay CV was 9.7%.

PON1 lactonase activity was measured using gamma-thiobutyrolactone (TBL) as substrate and Ellman's procedure was used to spectrophotometrically monitor (412 nm) the accumulation of free sulfhydryl groups via coupling with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) [27]. The reaction was run in the working buffer (50 mmol/L Tris, 1 mmol/L $CaCl_2$, 50 mmol/L NaCl, pH = 8), 50 mmol/L DTNB and 10.5 mmol/L TBL. A molar extinction coefficient of $13.6 \times 10^3\ L^{-1}\ mol^{-1}\ cm^{-1}$ was used for the calculation of enzyme activity, expressed in unit per liter. The intra-assay CV was 6.1% whereas the inter-assay CV was 9.8%.

2.3. Serum HDL cholesterol efflux capacity (CEC)

HDL CEC results analyzed in the present work belong to a previous published study [26]. Five cholesterol efflux pathways were evaluated by using specific cell models: total CEC, aqueous diffusion (AD)-CEC, ATP-binding cassette A1 (ABCA1)-mediated CEC; ATP-binding cassette G1 (ABCG1)-mediated CEC and scavenger receptor class B (SR-BI)-mediated CEC [28]; the methods were already been described [28]. Briefly, in all determinations cells were labeled with [1,2–^{3}H]-cholesterol and exposed to HDL, isolated from serum by polyethylene glycol. Serum HDL CEC was expressed as a percentage of the radioactivity released to the medium in 4 h (6 h for ABCG1-CEC) after over the total radioactivity incorporated by cells. Serum samples were determined in triplicate.

2.4. Vascular assessment

The determination of the following vascular parameters was performed in controls and HAL subjects:

2.4.1. Ultrasonography assessment

Vascular ultrasonography was performed by a single trained operator, blind to the patient general characteristics. Mean of common carotid intima-

media thickness (cIMT) was measured in triplicate bilaterally at the far wall of the distal common carotid artery (10 mm before bifurcation) in a location not containing plaques. Subclinical carotid atherosclerosis and sub-clinical femoral atherosclerosis were assessed.

2.4.2. Ankle-brachial index (ABI)

The ABI of each lower extremity, measured during the clinical examination with a handheld Doppler stethoscope according to a standardized protocol, was calculated by dividing the highest value of systolic blood pressure (SBP) of the posterior tibial or dorsalis pedis arteries by the highest SBP measured in both humeral arteries. A patient was considered to have PAD when the ABI was < 0.9 and arterial calcification with an ABI ≥1.4.

2.4.3. Flow-mediated vasodilation of the brachial artery

The measures were performed in a quiet room at a controlled temperature (24 °C), in resting subjects. Brachial artery was scanned in longitudinal section 2 cm above the antecubital fossa with B-mode ultrasonography images. The brachial artery diameters were calculated as an average of 3 consecutive measurements of the distance between the anterior wall and the posterior wall intima-lumen interface at end-diastole both before and 45 s after the deflation of a sphygmomanometer cuff, placed around the arm distal to the imaged artery segment, 60 mmHg above individual systolic pressure for 5 min. Flow-mediated vasodilation (FMD) of the brachial artery was calculated as the percent change in arterial diameter compared with baseline resting diameter.

2.5. Statistical analysis

Sample size was determined as previously described [26]. Novel variables were generated by normalizing lactonase or arylesterase activities for Apo A1 concentrations. By this mean, we calculated the specific activity of the enzyme, a measure of its concentration per unit of mass. The se and the other continuous variables were first analyzed for normal distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Variables not normally distributed were log transformed before entering statistical analyses. Group comparisons were performed using ANOVA (Sidak post-hoc for pairwise comparisons) for continuous variables and chi-square test for categorical variables. When it was possible, analysis of covariance (ANCOVA) was performed to test whether the differences revealed at univariate analysis were independent of potential confounding factors. Pearson's correlation coefficient was used to evaluate the possible association between PON1 activities and parameters of cholesterol efflux, vascular reactivity and damage. This test was followed by multiple regression analysis in order to check the independence of the observed simple associations.

The probability of having arterial plaques (Odds Ratio, O.R.; 95% confidence interval, C.I.—95%) in subjects with high arylesterase or high Apo A1 was calculated by multivariate logistic regression analysis. High/low arylesterase and ApoA1 serum levels were also combined into 3 groups in order to evaluate the risk of arterial plaques associated with the combination of these markers.

A two-tailed $p < 0.05$ was considered statistically significant.

Statistical analysis was performed using SPSS 22.0 software (SPSS, Chicago, IL).

3. Results

3.1. Clinical characteristics, lipid profile and vascular parameters in the sample groups

The main demographic, clinical and laboratory characteristics of the subjects enrolled in the study are summarized in Supplemental Table 1. HAL, HA, and controls did not significantly differ by prevalence of obesity and smoking status. Of note, HAL and controls, but not HA, were age- and gender-matched; HA differed from the other two groups as regards gender prevalence (Females: 5% vs. 90%), but not mean age. Conversely, HA group presented higher prevalence hypertension. Regarding lipid profile, patients with HAL had the lowest level of triglyceride (ANOVA, $p = 0.026$), and the highest levels total cholesterol (ANOVA, $p < 0.001$) and LDL-C (ANOVA, $p = 0.018$), while neither Apo B nor non-HDL-C significantly differ between the groups. The ratio unesterified/total cholesterol, although showing the highest values in HAL subjects (ANOVA, $p < 0.001$), was within the reference range for all the three groups (< 0.30) [29]. By definition, Apo A1 (ANOVA, $p < 0.001$), and HDL-C (ANOVA, $p < 0.001$) progressively increased from HA, to controls to HAL, according to selection criteria (Supplemental Table 1 and Fig. 1C and D).

As already shown and previously described [26], no differences were observed as regards plaque number (both in carotid and femoral arteries), ABI, cIMT, and FMD% between controls and HAL subjects (Supplemental Fig. 1).

3.2. PON1 activities in control, HAL and HA group

As shown in Fig. 1A, the trend of arylestarease and lactonase PON1 activities resembled that observed for HDL-C and Apo A1, but the differences were much less evident, with only HAL vs HA difference being significant ($p < 0.05$ for both comparisons). Considering the extent of changes in HAL group compared to controls, lactonase and arylesterase were increased by 15% and 17%, respectively (HDL-C and Apo A1 increased by 43 and 32%, respectively), while the decrease in HA was equal to 6% and 16% (HDL-C and Apo A1 decreased by 57 and 31%). As expected in light of HDL particles being the main carrier of PON1, the aforementioned differences in lactonase/arylesterase activities were no longer significant after adjustment for either HDL-C or Apo A1 (Supplemental Table 2); on the contrary, the adjustment for triglycerides and hypertension did not affect the differences in either of the PON1-actvities (Supplemental Table 3). Following the suggestion of Bergmeier et al. [30], we calculated the specific activity of PON1 (activity per HDL particle) by normalizing arylesterase/lactonase for the levels of Apo A1. Intriguingly, after this transformation, the trend of the two activities across the groups reverted (arylesterase, ANOVA: $p = 0.015$; lactonase, ANOVA: $p = 0.001$) with arylesterase/Apo A1 and lactonase/Apo A1 similarly increased by around 50–60% in HA compared to HAL (Supplemental Table 4).

3.3. PON1 activities and vascular parameters

None of the markers of early vascular damage evaluated (i.e. ABI, cIMT, and FMD) was significantly correlated with either arylesterase or lactonase activity (data not shown). On the contrary, the presence of established vascular lesion such as an arterial plaque was associated with a significantly ($p < 0.05$) lower level of arylesterase (but not of lactonase) (Fig. 2). Of interest, no significant association was observed between levels of Apo A1 or HDL-C and the presence of plaques (data not shown), despite their well-recognized athero-protective function.

Apo A1 is widely suggested to be the most important determinant of HDL function as well as a synergic and essential co-factor of PON1 [31,32]. Consistently with this proposed intimal connection between PON1 and this apolipoprotein, we found that they were strongly related to each other (arylesterase *vs.* Apo A1: r = 0.45, $p = 0.001$). To investigate whether this functional synergy could influence the occurrence of plaques, we compared the prevalence of these lesions with four possible combinations of high/low arylesterase and high/low (median = cut off) Apo A1 levels: 1) low arylesterase/low Apo A1 (n = 11), 2) high arylesterase/low Apo A1 (n = 9); 3) low arylesterase/high Apo A1 (n = 9), 4) high arylesterase/high Apo A1 (n = 11). As shown in Fig. 3, the group with low levels of both parameters presented by far the highest frequency (64%) of individuals with plaques; at the opposite, the group with High arylesterase/High Apo A1 included the lowest (18%). To check if these descriptive findings have statistical relevance, we performed a binary logistic regression, taking Low arylesterase/Low Apo A1 as reference group (Fig. 4). This analysis showed that high serum levels of either arylesterase or Apo A1 were not associated with a significant reduction in odds of having artery plaques (O.R: 0.21, 95% C.I.:

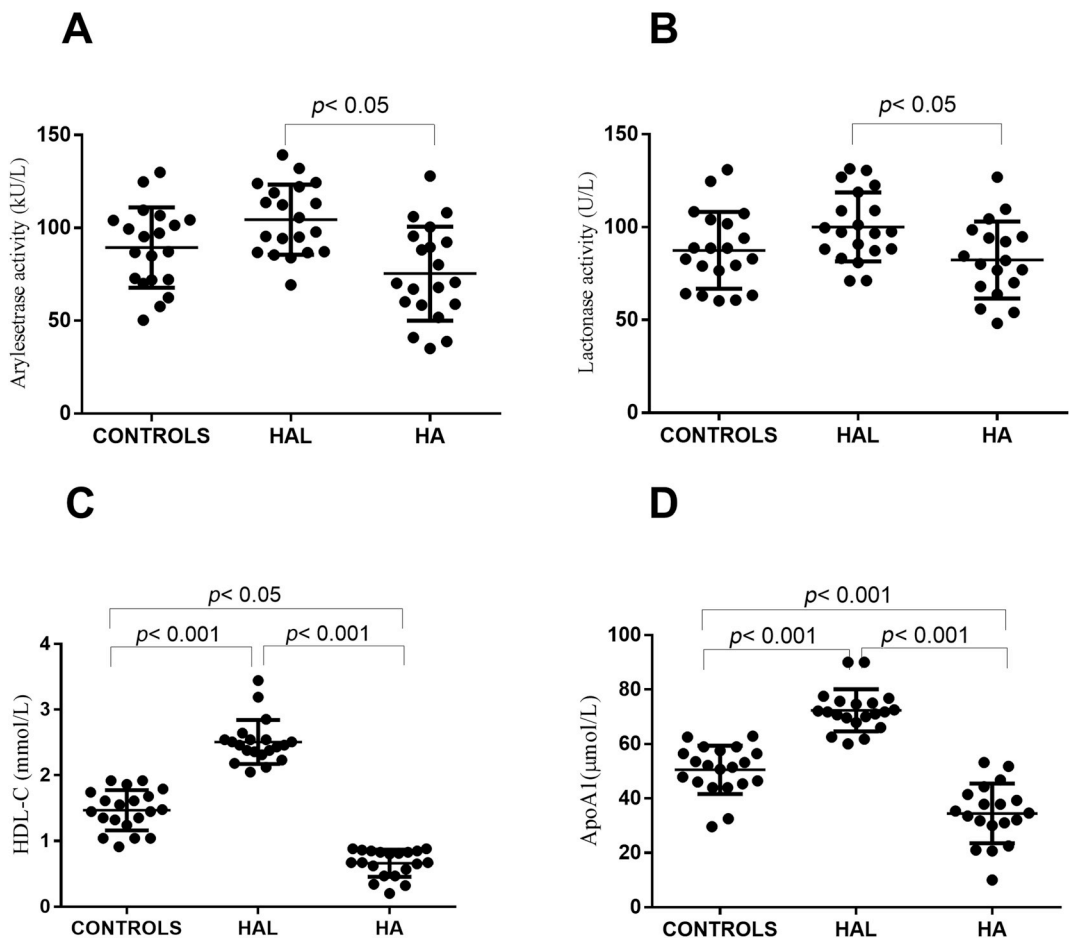

Fig. 1. PON1-arylesterase, PON1-lactonase, HDL-C and apoliprotein A1 in controls, HA and HAL.
(A), PON1-arylesterase; (B), PON1-lactonase; (C), HDL-C; (D), apoliprotein A1 (apo A1).

0.03–1.20; O.R: 0.14, 95% C.I.: 0.02–1.06, respectively). From the combination of the two dichotomous variables, it emerged that only the group with high levels of both arylesterase and Apo A1 showed a lower likelihood of having plaques compared to reference group (O.R: 0.38, 95% C.I.: 0.19–0.94: $p < 0.01$). Noteworthy, the strength of this association remained unaltered after controlling for potential confounders such as triglycerides and hypertension.

3.4. PON1 activities and cholesterol efflux capacity

As suggested by a wealth of in vitro evidence, the beneficial function of PON1 may be exerted by ameliorating the capacity of HDL to promote cholesterol efflux from cells [33,34]. To check this possibility, we evaluated the possible association between PON1 activities and the cholesterol efflux pathways measured in this study. We found that Arylesterase activity was positively correlated with AD-efflux, while lactonase was associated with both this pathway and total CEC (Table 1). As expected, Apo A1 emerged as a major determinant of all efflux pathways, and the aforementioned relationships observed for PON1 activities disappeared after adjusting for the levels of this apolipoprotein (Supplemental Tables 5 and 6).

4. Discussion

To the best of our knowledge, the present is the first study showing that HDL of HAL individuals may be relatively poorer in PON1 activities

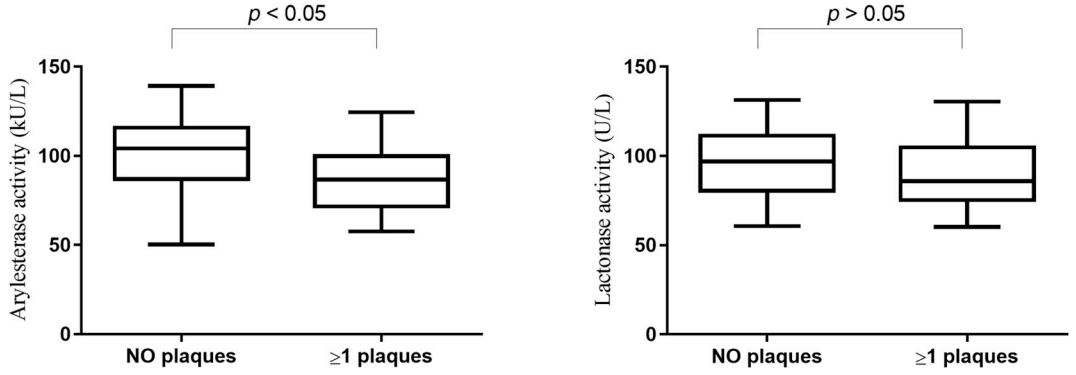

Fig. 2. PON1-arylesterase and PON1-lactonase levels in subjects with or without artery plaques.

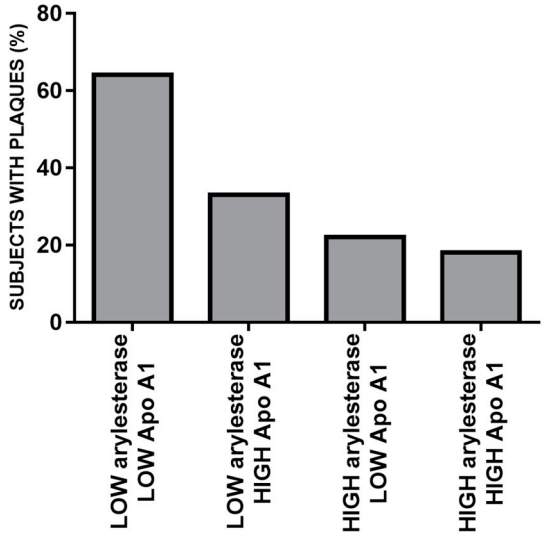

Fig. 3. Percentage of subjects with arterial plaques in groups with high/low levels of serum PON1-arylesterase and/or with high/low levels of serum Apoliprotein A1.
High/low levels of serum arylesterase and serum Apoliprotein A1 (Apo A1) were determined with respect to median values (arylesterase = 96 KU/L; APO A1 = 174 mg/dL).

compared to subjects with normal or very-low HDL-C levels. In turn, this low PON1 activity may reflect a minor efficiency of the lipoprotein, mitigating the potential atheroprotective effect due to the constitutively high concentration of HDL-C characterizing these individuals.

In line with our results, growing evidence suggests that HDL function may be more relevant than HDL-C concentration in protecting against CVD [35]. The antioxidant PON1 is widely suggested to be one of the components of HDL proteome mostly contributing to the lipoprotein atheroprotective function [33,36]. In line with these considerations, in vivo (mice over-expressing PON1) [15] and ex vivo (oxLDL pretreated with PON1) [37] studies suggest that PON1 stimulates cholesterol efflux from macrophages. HDL-mediated cholesterol efflux represents the first and the rate-limiting step of this complex and multi-step process. This recover of excess cholesterol from cells (including foam cells) can occur via passive/facilitated (AD-CEC and SR-BI-mediated) and active pathways (mediated by membrane proteins such as ABCA1 and ABCG1). A previous investigation conducted on the same controls and HAL groups, found that all cholesterol efflux pathways were comparable or only slightly increased in the latter group [26]. Notably, as observed for PON1, normalizing for Apo A1 reversed the trend, and all CEC pathways increased significantly in controls compared to HAL. Consistent findings from in vitro studies using recombinant or plasma purified PON1 [11,33,34], or the overexpressed protein in mice [15] showed that PON1 plays some role in CEC. Encouraged by these intriguing findings, we explored for the first time the possible association between serum PON1 activity and all CEC pathways. In apparent contrast with the proofs in support of an interaction of PON1 with ABCA1 [11,37], we did not find any association between cholesterol efflux mediated by neither this transporter nor by ABCG1 (mainly involved in cholesterol efflux to mature HDL [38]) and PON1 activities. However, lactonase (i.e. the activity putatively responsible of generating lysophosphatidylcholine) was weakly associated with both aqueous and SR-BI CEC pathways. As disclosed by multivariate analysis, this association was chiefly influenced by Apo A1, most likely because of the strong and well known [34] relationship of this apoliprotein with PON1 function and CEC of HDL. Overall, this result is consistent with the hypothesis that the contribution of PON1 in CEC may depend on its intimate functional and physical interaction with Apo A1 on the HDL suggested by the solved crystal structure of the enzyme [32].

Consistently with the mutual interaction between PON1 and Apo A1 [32,39,40], we found that: 1) the slight difference in PON1 activities

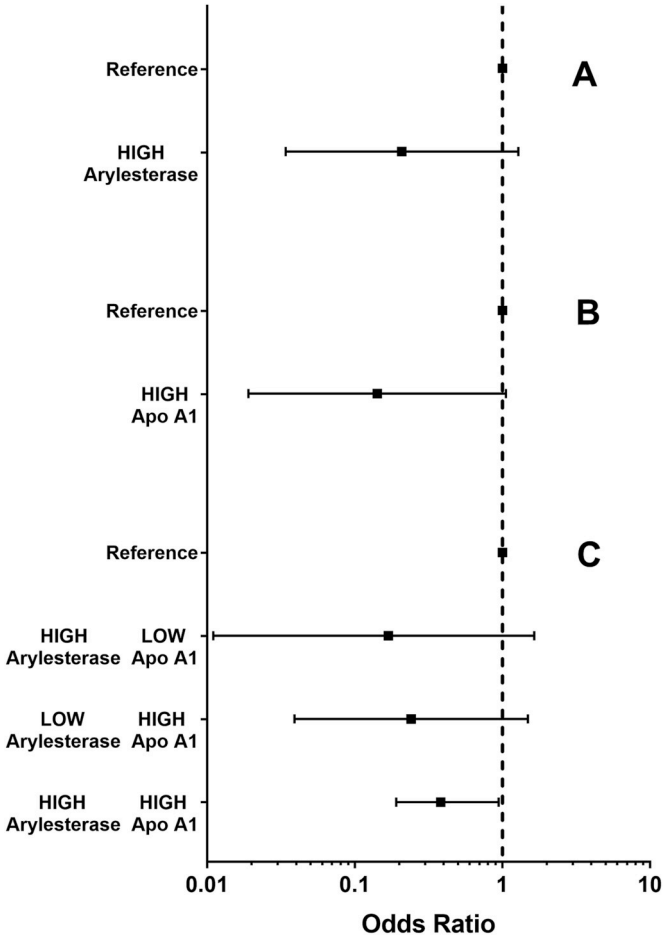

Fig. 4. Odds ratio (95% confidence interval) for the presence of arterial plaques in subjects with high/low levels of serum PON1-arylesterase and/or with high/low levels of serum Apoliprotein A1.
High/low levels of serum arylesterase and serum Apoliprotein A1 (Apo A1) were determined with respect to median values (arylesterase = 96 KU/L; APO A1 = 174 mg/dL).

across groups were dependent on Apo A1 (and HDL-C), but not on other potential confounders 2) high levels of arylesterase and Apo A1 are associated with less arterial plaques, in a way independent of age, gender, triglycerides and hypertension. The latter result is in partial agreement with some clinical/epidemiological studies, reporting an association between sub-clinical atherosclerosis and PON1 [41,42]. PON1 has been widely proposed to act, most likely in coordination with phospholipase A2 and vitamin E, as an antioxidant shield of Apo A1 [31,32]. This protective action is important for preserving the structural and functional integrity of Apo A1. Indeed, it has been observed that

Table 1
Correlation coefficients for the potential association of Apo A-1, PON1-arylesterase, and PON1-lactonase with serum HDL cholesterol efflux capacity through the different pathways (whole sample, n = 60).

	Apo A1 (r)	Arylesterase (r)	Lactonase (r)
Total efflux/4 h	0.670[a]	0.196	0.330[a]
AD-efflux/4 h	0.684 c	0.318[a]	0.355[a]
SR-B1-efflux/4 h	0.836[c]	0.024	0.330[a]
ABCA1-efflux/4 h	0.341[a]	−0.033	0.147
ABCG1-efflux/6 h	0.462[b]	0.046	0.043

[a] $p < 0.05$; $p < 0.01$;[c] $p < 0.001$.
AD, aqueous diffusion; SR-B1, scavenger receptor class B type 1; ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1.

oxidative modification primed by myeloperoxidase and other pro-oxidant agents seems to provoke an impairment of Apo A1 proprieties, affecting efflux capacity and anti-inflammatory function of HDL, thus favoring atherosclerosis development [39,43]. Consistently, Shao et al. found that patients with coronary artery disease have increased levels of oxidized Apo A1 [44].

Differently from us, other studies reported a significant, although generally weak, inverse correlation between PON1 activities and some early marker of artery diseases, such as cIMT [45–48]. However, these discrepancies might be due to differences in the design of the study, sample composition, as well as in the analytical procedures employed to assay PON1 activity. In particular, the exclusive assessment of paraoxonase activity, measured in two of these studies, is considered not suitable for epidemiological/clinical studies [17]. Indeed, this activity is influenced by PON1 polymorphisms (in particular 192 Q/R) much more than arylesterase and lactonase [17]. This is an important source of variability in PON1 data, which somewhat limits inter-laboratory comparisons.

The present study presents some caveats. First of all, our cross-sectional study design, as opposed to longitudinal approach, precluded our ability to establish any cause/effect relationship. Second, the small size of our sample and the lack of gender-match between the three groups (only HAL and controls were sex-matched as previously reported [26]) may affect the reliability of our conclusions. Third, since PON1 physically interacts with HDL particles, the measurement of enzyme activities on isolated HDL instead of whole serum sample, would be more valuable and informative. However, it is well established that, at least in healthy subjects, almost the totality of PON1 is associated with HDL [49]; thus, any alteration in its serum activity should mirror that of HDL-associated enzyme.

A fourth possible limitation of our study is related to the lack of a characterization of the HDL phenotype of our subjects, especially in terms of CETP and LCAT activity (but also other factors such as phospholipid composition, myeloperoxidase, oxidative status of Apo A1 etc.). However, a previous genetic analysis of our HAL subjects [26] showed a high prevalence of a CETP polymorphism associated to reduced CETP mass and activity [50]. In addition, the ratios unesterified/total cholesterol that we found in serum seem to suggest a normal cholesterol esterification process, reflex of physiological LCAT activity/mass [29].

Fifth, the method for lactonase activity assessment, though presenting important advantages over the others, has some limitations. Moreover HDL-associated PON3 elicits lactonase activity [12] and, even if presumably to a low extent (the enzyme and related activity, is two orders of magnitude less abundant than the other extracellular isoenzyme), it might interfere with the results.

In conclusion, our study showed that arylesterase and lactonase activities of PON1 are not significantly increased in individuals with HAL compared to controls. This unequal change in PON1 may account for the lack of benefit in terms of CVD risk reduction reported in HAL despite the important increase in their plasma HDL-C. This concept is indirectly supported by our finding of a significant inverse association between PON1 (together with Apo A1) and the presence of artery plaques. Further studies on larger sample are required to corroborate these preliminary findings.

Conflicts of interest

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

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Author contributions

Our work is a product of the intellectual environment of the whole team; all members have contributed in various degrees to its birth.

Conception and design of the study - AP, CC.

Acquisition of data - GBV, AP, CC, JMS.

Data analysis and interpretation - CC, AP, AT.

Drafting of the article - CC, AP.

Critical revision of the article - GBV, MLM, JMS, EDN, and GZ.

All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.04.218.

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