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SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1087–1094

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

The endothelial cholesterol efflux is promoted by the high-density lipoprotein anionic peptide factor

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Received 7 July 2004; accepted 23 March 2005

Abstract

The prevention of atherosclerosis depends on the high-density lipoprotein (HDL) capacity to stimulate the efflux of unesterified cholesterol (UC). We tested here the effects of 2 HDL apolipoproteins, apo A-I and the 7-kd anionic peptide factor (APF), on the UC efflux by human endothelial ECV 304 cells in culture. Apolipoprotein A-I (10 μmol/L) or APF (3.5 μmol/L) in lipid-free forms or small particles (13 nm with apo A-I or 19 nm with APF) were incubated in the presence of [4-^{14}C]UC. The phosphatidylcholines (PCs) were present either at a low level (0.35 mmol/L with apo A-I or 0.20 mmol/L with APF) or at a high level (1 mmol/L with apo A-I). We also tested either large 53-nm bile lipoprotein complex–like particles (3.5 μmol/L APF [13 μg/500 μL]) with a high PC level (0.65 mmol/L) or a 9-residue synthetic peptide (13 μg/500 μL), derived from the NH_2-terminal domain of HDL_3-APF, in a lipid-free or low-lipidated (0.20 mmol/L PCs) form. A control was developed in absence of the added compounds. A rapid [4-^{14}C]UC efflux mediated by APF added in free form or in 19-nm complexes was 2.2- to 2.3-fold higher than that mediated by apo A-I in free form or in 13-nm particles ($P < .05$). The level of this high APF-related efflux was comparable with that obtained with the 12-nm native HDLs (10 μmol/L apo A-I) or free PCs (1 mmol/L). The increase in the UC efflux was much more limited (1.4-fold) in the presence of the 53-nm APF/high-PC particles, but it was higher than that mediated by apo A-I. In addition, the efflux mediated by the synthetic peptide, in lipid-free or low-lipidated form, constituted the major part of that related to the full-length APF. Thus, all these particles are very active HDL components, able to act as cholesterol acceptors. Interestingly, we further showed a new anti-atherogenic property of APF as well as its metabolic importance and clinical relevance. By its involvement in the first step of the reverse cholesterol transport, APF could reduce the risk of cardiovascular disease.

1. Introduction

The concentration of high-density lipoprotein (HDL) cholesterol has been found consistently to be a powerful negative predictor of the premature coronary heart disease in humans [1]. The HDL phospholipids are efficient components against atherosclerosis, as their level correlates in a reverse manner with the severity of the angiographically defined coronary artery disease [2]. This beneficial action of HDLs involves pre-β–HDLs, primary forms containing only phospholipids and apolipoproteins such as apolipoprotein (apo) A-I (Euromedex, Mundolsheim, France) [3]. In the plasma of normolipidemic subjects, 4% to 10% of apo A-I is effectively carried by the pre-β–HDLs [1], particles able to promote the efflux of unesterified cholesterol (UC) from endothelial or fibroblast cells [4]. This efflux appears furthermore directly correlated with the level of HDL phospholipids [5], and it requires not only apo A-I but also other HDL apolipoproteins. Among those, the small 7-kd HDL anionic peptide factor (APF) might be a valuable candidate, being present at various concentrations in the 2 HDL_3 (6-13 μg/mL) and HDL_2 (3-6 μg/mL) subpopulations [6]. It shares 6 common epitopes with apo A-I, 3 of which are detected at the surface of native HDL

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doi:10.1016/j.metabol.2005.03.013

particles. This finding suggests structural and functional relationships between the 2 apolipoproteins [6]. Because APF exhibits a high binding affinity for lipids and is involved in the reverse cholesterol transport from the plasma toward the liver and bile [7], we hypothesized that APF could act as a physiological HDL interface, promoting the cellular cholesterol efflux.

Thus, this study was done to compare the levels of UC endothelial efflux mediated by the apo A-I and APF. The 2 apolipoproteins, either in lipid-free or lipidated forms, were incubated at their HDL concentrations. The low contents in phosphatidylcholines (PCs) are similar to those of native HDLs [8,9]. The high PC contents represent those found in plasmatic PC-enriched HDLs or in bile APF-enriched HDL-like particles, named the *bile lipoprotein complexes* (BLCs) [6]. Several peptides, analogues from class A, have been shown to mimic some properties of human apo A-I in vitro [10]. Thus, another analogue, a new synthetic peptide containing 9 residues, derived from the NH_2-terminal domain of HDL_3-APF, was also tested. It was used in a lipid-free or low-lipidated form to identify a possible structural element of APF able to stimulate the UC cellular efflux.

2. Materials and methods

Briefly, the UC efflux mediated by native HDLs or their components was measured at the level of endothelial cells previously loaded with low-density lipoproteins (LDLs) and [^{14}C]UC. The efflux was assessed by determination of the net movement of UC occurring from the cells to the culture medium and provided by the rapidly exchangeable pool of [^{14}C]UC.

2.1. Isolation of native human LDLs and HDLs

Blood of normolipidemic volunteers was collected after an overnight fast on EDTA and heparin lithium. Then, LDLs and HDLs were isolated in a sucrose solution by a sequential ultracentrifugation, at the buoyant density range of 1.021 to 1.063 and 1.063 to 1.210 g/mL, respectively. Low-density lipoproteins and HDLs were finally dialyzed 3 times at 4°C for 24 hours against a buffer containing 2 mmol/L Na_2HPO_4, 50 mmol/L NaCl, and 1 mmol/L EDTA (pH 7.4).

2.2. Anionic peptide factor isolation, antibody production, and quantitation

Anionic peptide factor was purified from human HDL_3 and isolated by a sequential ultracentrifugation at the density range of 1.193 to 1.206 g/mL and tangential ultrafiltration through miniultrasette devices (Filtron, Coignières, France). The supernatant was processed successively through 100- to 10-kd hydrophobic membranes. After concentration of the filtrate through a 3.5-kd membrane, the lipid-free APF was separated by a preparative high-performance liquid chromatography. The sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blot analyses showed that the purified APF was visualized in a 7-kd band. The preparation was free of any trace of cholesterol or phospholipid. Enzyme-linked immunosorbent assays were performed using a polyclonal antibody from immunized rabbits and a monoclonal antibody (Mab no. 32) from Balb/c mice that do not cross-react with apo A-I [6].

2.3. Obtention and preparation of the 9-residue synthetic peptide derived from the NH_2-terminal domain of APF

Some apo A-I antibodies identified as immunoglobulin G class 2F1, 5F6, and 4H1 (generous gift of Marcel et al [11]) were shown to cross-react with a cholesterol-binding (apo A-I [NWDSVTSTFSKL; residues, 49-60]) CNBr fragment 1 and with the full-length APF [6]. In our study, we tested a 9-residue synthetic peptide, containing the sequence EPGPVTTFT, as found in the NH_2-terminal domain of HDL_3-APF (peptide synthesized by Epitop, Nîmes, France). A stock solution (0.5 mg/mL) was prepared by dissolving the lyophilized peptide (purity of ~95%) in a 0.01 mol/L phosphate-buffered saline (PBS) (pH 7.4) and was stored at 4°C. The protein concentration was assessed by enzyme-linked immunosorbent assay, using the anti-APF antibodies [6].

2.4. Preparation of apolipoprotein/PC complexes

Complexes were prepared with polyunsaturated soybean PCs (Epikuron 200, containing more than 92% of PCs, Lucas Meyer, Hamburg, Germany) and either apo A-I or APF. After dissolution in pure ethanol and drying under nitrogen, the PCs were stirred in a 0.01 mol/L PBS for 30 minutes at 37°C. The apolipoprotein/PC complexes were then prepared in the absence of sodium cholate, by mixing apolipoproteins and different proportions of PCs (Table 1).

2.5. Electrophoretic mobility and size of the apolipoprotein/PC complexes

The characterization of reconstituted particles was investigated by electrophoretic mobility, with a nondenaturating 3% to 30% polyacrylamide gel, as described elsewhere [12]. The lipid staining was developed using the black Soudan B in a tris-hydroxymethylaminomethane–boric acid 27:73 (vol/vol) buffer (pH 7.6) (Lipophor assay, Bayer, Puteaux, France). The size of the different particles was measured at 26°C by quasi-elastic light scattering.

2.6. Incubation of ECV cells in culture

2.6.1. Cell culture

We tested the human endothelial cell line (ECV 304 cells) (generous gift of Pr. P. Bongrand [INSERM U. 387, Marseille, France]). The cells were grown until confluent in 24-well plates (Nunc, VWR International, Strasbourg, France). The culture was performed in a M199 medium

Table 1
Experimental design

	Control C	Native human HDLs	HDL components added in free form[a]				Apolipoprotein/low-PC complexes			Apolipoprotein/high-PC complexes[b]	
ApoA-I	–	10 μmol/L 150 μg/500 μL	10 μmol/L 150 μg/500 μL	–	–	–	10 μmol/L 150 μg/500 μL			10 μmol/L 150 μg/500 μL	
APF	–		–	3.5 μmol/L 13 μg/500 μL	–	–		3.5 μmol/L 13 μg/500 μL			3.5 μmol/L 13 μg/500 μL
Synthetic peptide[c]	–		–	–	13 μg/500 μL	–			13 μg/500 μL		
Low PCs	–	0.35 mmol/L 140 μg/500 μL	–	–	–	–	0.35 mmol/L 140 μg/500 μL	0.20 mmol/L 80 μg/500 μL	0.20 mmol/L 80 μg/500 μL		
High PCs	–		–	–	–	1 mmol/L 400 μg/500 μL				1 mmol/L 400 μg/500 μL	0.65 mmol/L 260 μg/500 μL

In all incubations, the medium contained 5% FCS. By its own HDLs, this serum provided 2.5 μmol/L apo A-I (37 μg/500 μL per well) and 0.087 mmol/L PCs (35 μg PCs/500 μL per well).

[a] In the experiment with HDL components, the ingredients (apo A-I or APF or the synthetic peptide in lipid-free form or PCs in free form) were added separately (4 different series).

[b] The complexes reconstituted with APF were BLC-like particles.

[c] The 9-residue synthetic peptide derived from the NH_2-terminal domain of APF was used to identify a structure element of APF able to stimulate the cellular cholesterol efflux.

containing 20% heat-inactivated fetal calf serum (FCS), 2 mmol/L glutamin, 100 U/mL penicillin G, and 100 μg/mL streptomycin (Gibco Life Technologies, Cergy Pontoise, France). The culture was maintained at 37°C, in a humidified atmosphere containing 95% oxygen and 5% carbon dioxide. When the cells were confluent, the culture medium was deprived from 20% to 5% of FCS. The addition of human LDLs occurred 24 hours later. The low level of FCS was unchanged throughout the experiment. Polymixin (Sigma, Saint Quentin Fallavier, France) was added at the concentration of 7 μg/mL.

We verified previously that the more appropriate experimental time required for LDL loading was 48 hours compared with 24 hours. When the incubations were carried out for 48 hours with decreasing doses of native human LDLs from 4.0 to 1.0 μmol/L apo B, the level of UC efflux significantly increased by 4-fold. Preloading the cells in absence of LDL led to a 2-fold decrease of the UC efflux (data not shown). Thus, to improve the UC efflux, the cells were preloaded in all experiments for 48 hours with native LDLs (1.0 μmol/L LDL–apo B [50 μg/500 μL per well] and 0.30 mmol/L of total LDL cholesterol [0.22 mmol/L of esterified cholesterol and 0.08 mmol/L of UC]) in the presence of 0.2 μCi [4-^{14}C]UC (50 mCi/mmol, NEN, Paris, France).

2.6.2. Test of cholesterol efflux

After incubation with [4-^{14}C]cholesterol and LDLs, the cells were washed once with 500 μL per well of 0.01 mol/L PBS containing 0.2% bovine serum albumin (pH 7.4) and twice with 500 μL per well of PBS (pH 7.4). The cells were then incubated for 1 hour with the medium containing 5%FCS. By its own HDLs, the above serum provided 2.5 μmol/L apo A-I (37 μg/500 μL per well) and 0.087 mmol/L PCs (35 μg PCs/500 μL per well).

The tested compounds were added as mentioned in Table 1:

1. no addition of compounds (control C, 500 μL per well);
2. addition of native human HDLs (10 μmol/L apo A-I [150 μg/500 μL per well]; 0.35 mmol/L PCs [140 μg/500 μL per well]);
3. addition of components in free form: lipid-free apolipoproteins (3.5 μmol/L APF [13 μg/500 μL per well] or 10 μmol/L apo A-I [150 μg/500 μL per well]), free PCs (1 mmol/L [400 μg/500 μL per well]), or the 9-residue synthetic peptide (13 μg/500 μL per well);
4. addition of apolipoprotein/PC complexes containing 2 different proportions of PCs:
   - the apo A-I/low PC or APF/low-PC complexes mimic native HDLs in the following proportions: (10 μmol/L apo A-I [150 μg/500 μL per well]/0.35 mmol/L PCs [140 μg/500 μL per well]; apo A-I/PC ratio of 1:35) or (3.5 μmol/L APF [13 μg/500 μL per well]/0.20 mmol/L PCs [80 μg PCs/500 μL per well]; APF/PC ratio of 1:57). We tested also the synthetic peptide/low-PC complexes (13 μg peptide/0.20 mmol/L PCs [80 μg/500 μL per well]; peptide/PC ratio of 1:57) (Table 1);
   - the apo A-I/high-PC complexes (10 μmol/L apo A-I/1 mmol/L PCs [400 μg/500 μL per well]; apo A-I/PC ratio of 1:100) mimic PC-enriched HDLs. The APF/high-PC complexes (3.5 μmol/L APF/0.65 mmol/L PCs [260 μg/500 μL per well]; APF/PC ratio of 1:185) mimic the BLCs (Table 1).

We chose to test only 2 proportions of PCs associated with apo A-I or APF. In fact, these complexes are interesting from a physiological standpoint, as they mimic particles really present in plasma [8,9] or bile [6] of healthy subjects.

At the end of all incubations, the [^{14}C] radioactivity was measured in each sample of the culture medium. The cells were rinsed (3 × 500μL PBS; pH 7.4) and lyzed with 240 μL per well of a 0.1 mol/L NaOH solution. We verified that the [^{14}C] radioactivity was exclusively carried by UC (97%) in cells and culture media.

2.7. Statistical analyses

The results were expressed as the arithmetical means of each group with their SEs. The statistical significance of the results was analyzed by a 1-way analysis of variance and the differences between groups were determined by the Fisher exact test ($P < .05$) using the Statview II Micro Computer Program (Abacus, Berkeley, Calif).

3. Results

The results were expressed in the relative UC efflux. This efflux was measured by the percentage of the [^{14}C] cellular load calculated after fixing at 100% the mean of the control values. In the control group, we observed a basal efflux related to the presence in the culture medium of HDLs provided by the FCS that was added for cell growing. This non-negligible efflux represents the fourth of that subsequently obtained after addition of native HDLs.

3.1. Properties of apolipoprotein/PC complexes and native HDLs

We determined the properties of apolipoprotein/PC complexes containing either apo A-I or APF. They showed pre-β electrophoretic mobilities. Their size distribution (data not shown) was 13 nm for the apo A-I/low-PC complexes and 19 nm for the APF/low-PC complexes, respectively. The APF/high-PC particles consisted of larger particles with a diameter of 53 nm. The size of native HDLs was 12 nm.

3.2. Cholesterol efflux in the presence of native human HDLs

After preloading the cells with LDLs, a substantial UC efflux was obtained when HDLs were incubated for 40 minutes. This efflux was time dependent and was significantly enhanced, in comparison with the control (data not shown). We observed that the UC efflux was HDL dose-dependent. It increased linearly and significantly by 38%, when the HDL–apo A-I increased from 3.3 to 10 μmol/L. Such findings signify that the process was not saturated at this concentration range, and we chose the maximal concentration of apo A-I (10 μmol/L [150 μg/500 μL per well]) and the maximal HDL_3-APF level (3.5 μmol/L [13 μg/500 μL per well]) [6] in the following experiments. The UC efflux mediated by the native HDLs was largely and significantly higher ($P < .05$) than the control efflux (Table 2).

3.3. Cholesterol efflux in the presence of lipid-free apolipoproteins

Apolipoprotein A-I and the control induced comparable UC efflux (Table 2). The apo A-I–dependent efflux represented no more than 50% ($P < .05$) of the efflux mediated by the native HDLs (Table 2). The APF-related efflux was significantly increased by 2- to 2.3-fold ($P < .05$) compared with the efflux obtained in control conditions or in the presence of apo A-I. The APF-related efflux was established at a level comparable with that obtained with the HDLs (Table 2).

Table 2
Endothelial [4-^{14}C] relative cholesterol efflux by native human HDLs or by apo A-I, APF or PCs added in free form or in complexes

Components[a]	Relative efflux	Statistical significance ($P < .05$)
1. Control	100 ± 3.7	
2. Native HDLs	210.8 ± 5.4	vs 1
3. PCs[b]	196.3 ± 18.8	vs 1, 4, 5, 6, 9
4. Apo A-I[b]	97.9 ± 7.1	vs 2
5. Apo A-I + low PCs[c]	89.8 ± 4.6	vs 2
6. Apo A-I − high PCs[c]	108.2 ± 7.4	vs 2
7. APF[b]	219.3 ± 9.2	vs 1, 4, 9
8. APF + low PCs[c]	233.4 ± 25.4	vs 1, 5, 9
9. APF + high PCs[c]	138.9 ± 8.1	vs 2, 7
10. Synthetic peptide[b]	188.7 ± 7.1	vs 1, 4
11. Synthetic peptide + low PCs[c]	146.5 ± 19.8	vs 5, 8, 10

Values are means with their SEs (n = 6 for each group). Significance was analyzed by 1-way analysis of variance and the difference was characterized by a superscript corresponding to the number allowed to each component. The relative cholesterol efflux was determined by the percentage of the [^{14}C] cellular load calculated for a mean of the control value of 100%. Cell culture was performed after a 48-hour load with 1 μmol/L apo B of native human LDLs, in the presence of 0.4 μCi/mL [4-^{14}C]UC.

[a] In all incubations, the medium contained 5% FCS. By its own HDLs, this serum provided 2.5 μmol/L apo A-I (37 μg/500 μL per well) and 0.087 mmol/L PCs (35 μg PCs/500 μL per well).

[b] Free form.

[c] Apolipoprotein/PC complexes.

3.4. Cholesterol efflux in the presence of PCs in free form

The level of UC extracted by the free PCs was significantly increased, up to 2-fold ($P < .05$), compared with that mediated by lipid-free apo A-I or by the control. This level was comparable with that obtained with native HDLs or lipid-free APF (Table 2).

3.5. Cholesterol efflux in the presence of apolipoprotein/PC complexes

The UC removals by either 10 μmol/L apo A-I/0.35 mmol/L PCs or 10 μmol/L apo A-I/1 mmol/L PCs or lipid-free apo A-I were not significantly different (Table 2).

There was no significant difference between the large UC efflux mediated by the 3.5 μmol/L APF/0.20 mmol/L PC complexes and the lipid-free APF (Table 2). This high value of efflux was comparable with that obtained with native HDLs or free PCs. The UC efflux promoted by APF/low-PC complexes was significantly higher ($P < .05$) than that obtained with the apo A-I/low-PC complexes. It decreased significantly ($P < .05$) when the PC level increased from 0.20 mmol/L to 0.65 mmol/L (Table 2).

3.6. Cholesterol efflux in the presence of the 9-residue synthetic peptide derived from HDL_3-APF

The 9-residue synthetic peptide in lipid-free form exhibited a very active effect on the UC efflux, representing a large proportion of that mediated by the full-length APF in free form. The peptide/low-PC complexes promoted the cholesterol efflux with a higher efficiency ($P < .05$) than the apo A-I/low-PC complexes (Table 2). However, the synthetic peptide/low-PC particles exhibited a reduced efficacy ($P < .05$), in comparison with the lipid-free peptide or APF/low-PC complexes (Table 2).

4. Discussion

The aim of this present study was to investigate the endothelial cholesterol efflux mediated by HDL–apo A-I, -APF, or -PCs, incubated in free form or in apolipoprotein/PC complexes containing low or high PC levels found in human plasma [8,9] or bile [6].

The primordial role of the HDL apolipoproteins in cholesterol efflux is largely described. Nevertheless, our present observations showed that no more than half of the cholesterol efflux to HDLs was apo A-I–dependent. We further investigated the efflux capacity of APF, which is another HDL apolipoprotein previously described as a cholesterol carrier [7]. We observed that the APF-related efflux was 2 times higher compared with that mediated by apo A-I, APF being added in free form or in APF/low-PC complexes. These complexes are interesting from a physiological standpoint because they constitute plasmatic

precursor of mature HDLs containing APF. These HDLs, isolated at the buoyant density range of 1.193 to 1.206 g/mL, are effectively present in plasma of humans [6] or animals [8].

In fact, some homologies of apo A-I and APF occur by 6 common epitopes, 3 of which being detected at the surface of intact HDL particles [6]. These structural similarities account for the fact that both apolipoproteins can stimulate the cholesterol efflux to some extent (50% of the total HDL-dependent efflux). In addition, we observed here that the synthetic peptide containing the sequence EPGPVTTFT, either in a lipid-free form or in peptide/low-PC complexes, constitutes a structural element of APF that promotes efficiently and substantially the UC cellular efflux.

Thus, we advance that the other 50% of the APF-related efflux were apo A-I–independent. This important APF-mediated process depends on the physicochemical characteristics of the apolipoprotein. By its highly tensio-active property, APF is able to deeply penetrate into phospholipid monolayers at a higher surface pressure, compared with the capacity of apo A-I [13]. A relation exists between the exclusion pressure and the property of apolipoproteins to stimulate the phospholipid efflux from membranes such as those of fibroblasts [13]. A consequence is effectively the promotion to a great extent of the cellular UC removal mediated by APF.

The score of cholesterol efflux obtained in the presence of HDL apolipoproteins should largely depend also on their conformation. We observed here that the monomeric form (7 kd) of APF into plasmatic HDL-like structures was very efficient in cholesterol efflux. Previous findings clearly showed that the monomeric form of APF was secreted into the bile, where a self-association induced by an ionic strength resulted in an homodimeric form, the 14-kd calcium-binding protein [7]. By contrast with APF, the calcium-binding protein has been described to be unable to promote the UC efflux from fibroblasts in culture [14]. Such discrepancies between the answers of the 2 forms may be related to their differences in their lipid binding affinity, as advanced elsewhere to explain some changes in protein conformation and functions in lipid metabolism [15,16].

Our contrasting findings concerning the cholesterol efflux mediated by either apo A-I or APF should be interpreted in the light of other findings, demonstrating that only a slow and constant rate of cholesterol removal is controlled by apo A-I [17]. A rapid efflux, through a non-ABCA1 process [18], implies unidentified proteins different from apo A-I [17]. This ABCA1-independent efflux may represent up to 80% of the total efflux, as observed elsewhere at the level of vascular cells [19]. The scavenger receptor BI (SR-BI), efficient to prevent atherosclerosis [20], may be largely involved in the rapid UC efflux. Previous findings showed that APF is a physiological ligand of SR-BI at the intestinal level [21]. Thus, we advance that APF can be one of the protein candidates, related to the rapid UC efflux dependent on SR-BI.

The role of HDL phospholipids during the cholesterol efflux is furthermore underlined [9]. Previous studies developed in humans or in animals emphasized the effect of dietary PCs that reinforce the HDLs and the reverse cholesterol transport [22-26]. The HDLs act as acceptors of the desorbed cholesterol from cell membranes [27]. This process is largely stimulated in vitro by addition of PCs in free form, able to stimulate the UC cellular efflux [5,28,29], and by fusion of these PCs to the membranes. The mechanism is probably of physiological relevance, because in our present study, the high score of the PC-mediated efflux is comparable with that observed with native HDLs. Our deduction is reinforced by the fact that phospholipid-enriched HDLs are largely related to SR-BI [30], whereas the phospholipid-poor forms are more strikingly related to ABCA1 [9,31]. Interestingly, a minimal phospholipid level is therefore required to initiate the basal cholesterol efflux mediated by apo A-I through ABCA1 [32,33]. Zhao et al [32], using particles with a PC/apo A-I ratio no more than 35:1, suggested that the cholesterol efflux varies substantially and is directly correlated with the concentration of phospholipids present in the particles. Nevertheless, we observed a smaller increase by only 20% in the cellular efflux, when the PC/apo A-I ratio increased from 35:1 to 100:1. Thus, a saturation of the efflux occurred at the highest PC/apo A-I ratio. Such findings are not surprising, seeing that the ligands for ABCA1-mediated efflux are poorly lipidated [9,31]. Given this background, and considering the 5- to 7.7-nm particles described by Zhao et al [32], we note the genesis of larger 13-nm structures. All these differences are consistent with the observations of Davidson et al [34], showing the lower efficiency of the large particles compared with the small particles as acceptors of cellular cholesterol. More prominently, Yancey et al [9] demonstrated that the higher phospholipid/apo A-I ratios, related to the higher HDL size, correspond to the lower and minimal ABCA1 efflux, as effectively observed in the present study.

Nevertheless, the UC removal promoted by particles containing APF was much higher than that mediated by particles containing apo A-I, whatever the proportions of PCs. The increase of the PC/APF ratio from 57:1 to 185:1 produced particles evolving from 19 to 53 nm, still efficient in the UC efflux. An explanation for the large and rapid SR-BI–related efflux is that the ligands are fully lipidated lipoproteins rich in phospholipids [30], even in the case of the bile APF-enriched BLC [6,35].

A relevant interpretation of the differences of efflux mediated by apo A-I and APF is that APF was substituting for apo A-I in HDLs, when the PC concentration increased and exceeded the PC holding capacity of apo A-I. Thereafter, the APF-containing particles continued to be active and evolved from the HDL to BLC structures, simultaneously with the increase in the phospholipid/APF ratio and the enlargement of the particle size [6]. However, the 53-nm APF/high-PC, BLC-like particles [35] exhibited a lower capacity of efflux, in comparison with the 19-nm APF/low-PC

particles. We advance that a moderate down-regulation of SR-BI by the high PC levels could explain this decrease in the APF-related efflux.

In conclusion, a new anti-atherogenic property of APF in lipid-free form or in association with PCs into HDLs is clearly underscored for the first time in this study, by using the in vitro model of endothelial cells in culture. Anionic peptide factor and its structural 9-residue peptide efficiently favor the cellular cholesterol efflux.

Our findings constitute an interesting step in the research of relevant biomarkers of prevention or regression of atherosclerosis, such as APF, by its metabolic importance and clinical relevance. The prophylactic effect of dietary PCs, through the reinforcement of HDLs and the amplification of APF expression, may represent a new target in the treatment and prevention of the cardiovascular disease.

Acknowledgment

The authors thank Nathalie Angelini for her expert technical assistance.

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