Association of endothelial function and vascular data with LDL-c and HDL-c in a homogeneous population of middle-aged, healthy military men: Evidence for a critical role of optimal lipid levels

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Abstract

Background: Modifying lipids levels underpins atherosclerosis prevention. Flow-mediated dilation (FMD) could advise which patients to treat and to what extent. Little is known about the influence of near-normal lipid levels on the endothelium and the mechanisms related to different lipid fractions. We studied associations between FMD and lipids, focusing on normal lipid levels.

Methods: An age-homogeneous sample of 171 healthy, untreated military men (mean age 35.5±1.1 years) was studied: serum lipid determination and brachial artery ultrasound with a forearm ischemia cuff and automated measurement were performed. NCEP-ATP III groups were used.

Results: Significantly smaller vessel diameters were found among individuals with high HDL-c (4.10 mm vs. 4.24 mm), optimal LDL-c (4.00 mm vs. 4.22 mm), and normal triglycerides (<150 mg/dl) (4.15 mm vs. 4.31 mm). Basal diameter correlated significantly with HDL-c and triglycerides. There were significant differences in FMD between low HDL-c compared to the rest (4.13% vs. 5.07%) and between optimal and near-optimal LDL-c compared to the rest (5.28% vs. 4.56%). HDL-c and LDL-c correlated with FMD. The inverse relation of high LDL-c and FMD is partially due to a decreased stimulus. Besides, stimulus heterogeneity may mask HDL-c link with FMD.

Conclusion: Those subjects naturally (not pharmacologically) in the healthy tail-end of the lipid distributions have the best endothelial function and smaller vessels. Functional vascular remodeling might precede anatomical remodeling and, in early stages, vessel size should be considered a risk indicator rather than an atherosclerotic sign. Furthermore, controlling the stimulus seems necessary for detecting the relationship between HDL-c and FMD, and should be performed regularly.

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1. Introduction

Endothelial dysfunction has been described as the intermediate link between cardiovascular risk factors and the development of atherosclerosis [1,2]. When present, endothelial responses are impaired all over the vascular tree. It is also believed that microcirculation and conduit vessels are both affected when endothelial dysfunction is present [3] and that both vascular beds could be equally used for exploring endothelial health. Nevertheless, atherosclerotic clinical
diseases occur mainly in conduit vessels; therefore, different responses to pro-atherosclerotic factors of the endothelium in conduit and in microcirculation vessels might be relevant. Flow-mediated dilation (FMD) is a widely used method \[4\] that reflects endothelial dysfunction in conduit arteries.

Modifying lipids levels underpins atherosclerosis prevention. FMD could advise which patients to treat and to what extent. An association between lipid levels and endothelial function has been described, but there are few studies dealing with the influence of normal and near-normal lipid levels on the endothelium in non-treated subjects. Very low LDL-c levels in coronary patients under treatment associate with an increased FMD \[5\], but in general research has been focused on the opposite extreme of lipid values \[6–8\].

We determined the association of low LDL-c levels and high HDL-c levels with endothelial function in healthy subjects. We explored it in terms of FMD and other related vascular parameters. We also explored lipids’ influence when considering FMD in relation to flow stimulus.

2. Methods

A cohort of military men underwent a thorough preventive cardiovascular examination in the AGEMZA study \[9,10\]. In this group, the population’s age was around 35 years. Participants with major clinical disease or undergoing pharmacological treatments were excluded from further analysis (two subjects, one for each reason). All subjects gave written, informed consent to participate.

This was a cross-sectional observational study. Health status and previously known diseases, habits, and family history were obtained through a questionnaire. Weight, height, and blood pressure were measured in accord with current quality standards. Fasting serum samples were obtained from the subjects and analyzed in the University Clinical Hospital “Lozano Blesa” (Zaragoza, Spain). Analyses were performed for total cholesterol, triglycerides, and HDL-c by standard enzymatic laboratory techniques. LDL-c was calculated using the Friedewald formula \[11\] in subjects whose triglycerides levels were less than 400 mg/dl.

2.1. Endothelial function testing

Peripheral endothelial function was assessed using brachial artery ultrasound with a flow-mediated dilation protocol similar to that originally described by Celermajer et al. \[6\]. This followed the main guidelines of the International Brachial Artery Reactivity Task Force \[4\] and the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension \[12\]. Longitudinal images of the brachial artery above the antecubital fossa were continuously recorded at baseline and through vasodilator stimuli. Distal ischemia was induced by means of a pneumatic cuff inflated in the forearm (downstream of the studied portion of the vessel) to suprasystolic pressure for 5 min. Eight minutes later, sublingual nitroglycerin (25 μg administered in solution with a pipette) was used as an endothelium-independent vasodilator.
Images of the brachial artery were obtained with a multifrequency (5.5–7.5 MHz) linear-array vascular transducer on a Hewlett-Packard Sonos 4500 Imaging System (Agilent Technologies Inc., Andover, Massachusetts, USA). Vessel blood velocity was monitored with pulse-wave Doppler interrogation to detect reactive hyperemia. The images of the vessel and the Doppler spectrum were digitized in end-diastole using a frame grabber.

A computerized image analysis method was used on the image sequences [13,14]. The method is based on pair-wise image matching (registration). Registration provides a scaling value that models the transversal deformation of the artery occurring between two points in time. This method is more robust to speckle noise than other edge-based techniques and assesses the vasodilation curve more precisely than other automated methods. To obtain a curve of absolute values (Fig. 1), the diameter of the initial artery image of each study was measured with a computerized tool [13] by manually fitting a curve to each vessel wall and calculating the mean distance between them. For each vasodilation curve, two reference baseline diameters were established: one before vasodilation in a region free of motion artifacts selected just after cuff release, and another in the region of the curve prior to the administration of nitroglycerin. Flow-mediated and nitroglycerin vasodilations were calculated as the peaks of diameter change measured as a percentage of baseline diameters [4].

From the Doppler velocity distribution chart provided by the echograph, we acquired time-averaged maximum velocity measurements throughout the test (Fig. 1), using an automated technique similar to that described by Tschirren et al. [14,15]. Basal blood velocity was defined as the average velocity during the initial minute at the beginning of the test. Peak hyperemia blood velocity was defined as the maximum velocity after cuff release. Average hyperemic blood velocity was defined as the average velocity during the first minute of hyperemia and used to evaluate the stimulus.

2.2. Statistical analysis

Data were analyzed with SPSS (Version 10.0, SPSS, Inc., Chicago, Illinois). Mean comparisons (t test) and Pearson’s correlation were computed to demonstrate associations between FMD and lipid fractions. Multivariate linear

![Fig. 2. Average basal artery diameter for each one of the National Cholesterol Education Program’s lipid categories and its 95% confidence interval. Those figures at the bottom of the bars are the depicted average values and those above the graphic the number of subjects in each category.](image-url)
regression analysis was employed to model FMD, controlling for the hyperemic stimulus. We divided the cohort into groups according to lipid levels as defined by the National Cholesterol Education Program Adult Treatment Panel III [16] in order to compare FMD and other vascular parameters (basal diameter and basal blood velocity) among them. In all analyses, statistical significance was assumed when \( p < 0.05 \) (two-tailed analysis). Statistical tests were conducted on transformed variables (square root of FMD, abbreviated \( \text{sqrFMD} \); and logarithm of triglycerides) in those variables that did not have normal distributions. In this article, continuous random variables are characterized as mean \( \pm \) standard deviation.

3. Results

3.1. Sample characteristics

A total of 171 subjects agreed to participate, satisfied the inclusion criteria, and fulfilled the imaging protocol for FMD. Their characteristics are reported in Table 1. The sample corresponds to a population with favorable lipid levels, so pathological extreme values were scarcely represented ("n" values of Figs. 2 and 3). Among them, only 8% had high blood pressure. For those parameters with non-normal distributions, medians and interquartile ranges were: triglycerides 103 (69) mg/dl and FMD 4.43 (2.79)\%.

3.2. Relationship between flow-mediated dilation and lipids

Lipid levels were associated with basal vascular conditions. Arterial diameter tended to be smaller under better lipid conditions, as can be seen in Fig. 2. We split the sample into two groups according to thresholds derived from the figure for each lipid fraction. Individuals with HDL-c > 60 mg/dl (high) showed significantly smaller diameters (4095 \( \mu \)m vs. 4237 \( \mu \)m; \( p = 0.033 \)) than the rest. Individuals with LDL-c < 100 mg/dl (optimal) showed significantly smaller diameters too (4001 \( \mu \)m vs. 4223 \( \mu \)m; \( p = 0.007 \)). The same happened for subjects with triglycerides < 150 mg/dl (normal) (4149 \( \mu \)m vs. 4311 \( \mu \)m; \( p = 0.035 \)). Accordingly, a significant linear correlation was found for basal diameter with HDL-c \( (r = -0.163; p = 0.033) \) and with the logarithm of triglycerides \( (r = 0.234; p = 0.002) \). Basal flow, represented by basal blood velocity, also tended to rise as the lipid profile worsened (graphs are not shown) but no significant differences were
found when comparing basal flow of the optimal groups with the rest of the sample.

Fig. 3 shows that FMD was also related to total cholesterol and its fractions and presented a gradient. Again, we split the sample into two groups according to thresholds derived from the figure for each lipid fraction. There were significant differences between low HDL-c compared to the rest (means: 4.13% vs. 5.07%; medians: 3.77% vs. 4.49%; sqFMD t test: \( p=0.042 \)) and between optimal and near-optimal LDL-c compared to the rest (means: 5.28% vs. 4.56%; medians: 4.81% vs. 4.02%; sqFMD t test: \( p=0.013 \)). Furthermore, a significant linear correlation was found for sqFMD with HDL-c (\( r=0.163; p=0.034 \)) and with LDL-c (\( r=-0.201; p=0.008 \)).

3.3. Multivariate analysis

In the FMD test, endothelial function is assumed to be related to the vasodilator response of endothelium after a standard flow stimulus. However, intensity of the actual stimulus is heterogeneous among subjects. This, represented by average hyperemic blood velocity (dependent on small vessels’ response to ischemia) correlated linearly with sqFMD (\( r=0.369, p<0.001 \)).

We adjusted for average hyperemic blood velocity in a linear regression model for sqFMD explained by LDL-c. Its standardized beta coefficient decreased from −0.201 to −0.169. Conversely, the coefficient of HDL-c increased from 0.163 to 0.205 after accounting for blood velocity in the bivariate model. In both cases, the lipid fraction under consideration remained a significant term after adjustment.

Interestingly, a model trying to explain square-root FMD with both cholesterol fractions, presented HDL-c as non-significant. After controlling for the blood velocity, the importance of the fractions reversed: HDL-c was significant and LDL-c was not. Finally, we noticed that adjustment for BMI did not change any of the described models.

4. Discussion

We observed significantly different FMD for individuals with low HDL-c compared to the rest and also different FMD for individuals with optimal and near-optimal LDL-c compared to the rest. A coherent progression was evidenced along the range of lipid levels, and cholesterol fractions correlated significantly with FMD.

A relationship between FMD and HDL-c and LDL-c has already been shown, describing mainly differences between patients with extreme levels versus the normal population. Most studies are based on pooled samples combining high-risk or diseased individuals (diabetes [17], coronary heart disease [18,19], hypercholesterolemia [20], hypertryglyceridemia [21], hypoalphalipoproteinemia [22]) and normal individuals, or even samples entirely made up of high-risk individuals (obese [23], hyperlipidemic [24], suspected coronary heart disease [8,25,26]). These scenarios render higher correlation coefficients than those studies that, like ours, are based on a more homogeneous and healthier population. The only study that is similar to ours is that of Jensen-Urstad et al. [27] and it reports moderate coefficients. Thus, the modest value of the correlation coefficients in our study, when compared to those previously reported, can be due to a shortened range of lipid levels and to a small number of extreme pathological values. In addition, our sample was restricted to men with a narrow age range, which may further reduce the variability of FMD in the sample.

A trial with statins, performed on a small sample with LDL-c levels <160 mg/dl, showed endothelial function improvement in healthy subjects [28]. Reaching very low levels in coronary patients undergoing treatment with statins also rendered better endothelial results [5]. In our study, subjects with spontaneously low LDL-c levels also presented better endothelial health. This reassures these levels as therapeutic goals, independently of the drug used to reach them. The same trends could be observed for HDL-c.

The presence of wider vessels has been described in high-risk [8] and atherosclerotic [29,30] individuals. In 55 year-old individuals, common carotid artery diameter correlated significantly with total cholesterol, HDL-c, and triglycerides [30]. In spite of this, until now there was no evidence that this relationship between diameter and lipids also applies to normal and optimal lipid levels in middle-aged people. We have found just this evidence for HDL-c, LDL-c, and triglycerides.

An expansive atherosclerotic artery remodeling has been described by other researchers [31]. However, before that anatomic change occurs, some sort of toxic or compensatory reactive vasodilation must exist in patients with high atherosclerotic lipid levels. Furthermore, differences appear in vessel size soon after losing the theoretically optimal (near-fetal) levels. Hence, vessel size alone should be considered a risk indicator rather than an atherosclerotic signal as previously suggested [29]. Vessel size could be a marker of endothelial dysfunction. In dyslipidemia, compensatory mechanisms could activate the nitric oxide pathway even in absence of shear stress; therefore, reserve regulatory capacity would be reduced when physiological stimuli appear. This hypothesis will have to be confirmed by future research. Probably similar changes happen in resistance arteries.

FMD changes with gender and age [32]. Usually, this makes it difficult to discover subtle associations. Strategically, our sample was homogeneous in terms of these variables and we could focus multivariate analysis in controlling the stimulus of the test, thus going one step further than previous work. Our cross-sectional study is not conclusive for causality but we hypothesize here such a connection to discuss results from multivariate analysis. Controlling for the hyperemic stimulus increased HDL-c association strength with FMD but decreased that of LDL-c. This finding certainly suggests that both lipids exert their influence on the single parameter FMD through different mechanisms.
We observed that LDL-c levels importantly influence FMD, but part of this effect is through changes in microcirculation’s response to ischemia, that is to say, in reactive hyperemia. Reactive hyperemia also represents endothelial health to some extent [33], but in a different vascular territory. After controlling this stimulus, HDL-c took over influencing FMD. Therefore, HDL-c seems to actually have an impact on the intensity of the endothelial response to increased shear stress in conduit vessels. To explore these HDL-c influences, therefore, hyperemia should necessarily be measured, as heterogeneity in the stimulus may mask the HDL-c link with FMD. Moreover, it is not possible to detect them [34] using the endothelial explorations based exclusively on measuring post-ischemic reactive hyperemia, such as post-ischemic finger measurements [35]. In the same way, also some variants of FMD, like upstream cuff [36], probably blunt this association because dilation also depends on ischemic mediators.

Other mechanisms have been described for the HDL-c effect on FMD. Association of nitroglycerin-mediated dilation with HDL-c was described by previous studies [8] and a reduced smooth muscle sensitivity associated with low HDL-c was also observed in diabetic patients [37]. Hints to association between HDL-c and nitroglycerin-mediated dilation were observed in our analysis (graphs are not shown), but the differences were not statistically significant. One more possible mechanism would be a reduced reserve of the nitric oxide system due to an increased basal endothelium activation [38], as we mentioned before.

4.1. Conclusions

To summarize our study: better vascular health, in terms of endothelial function, was found among those subjects naturally (not pharmacologically) in the healthy tail-end of the lipid distributions, suggesting that at least part of the endothelial health improvement described for aggressive lipid-lowering drugs can be explained by their effect on lipids. In addition, it was remarkable that cholesterol fractions and triglycerides had a strong influence on vessel diameter even in a healthy population. Favorable levels were associated with smaller arterial diameters. This discovery has pathophysiological implications and deserves further research. Moreover, we observed differential influences of cholesterol fractions on vascular function and it will be necessary to further investigate pathophysiology of these differences. Our work also established that controlling the flow stimulus seems necessary for detecting the relationship between HDL-c and FMD, and should be thus performed regularly.

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