Original Research

Effects of Lipid Profile on Forearm Hyperemic Response in Young Subjects

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Introduction: The role of lipids in atherogenesis is now well established. However, the exact mechanisms by which different lipoproteins affect endothelial function and induce atherogenesis are still not well understood. In the present study we examined the effect of lipid profile on forearm vasodilatory response to reactive hyperemia, an index of endothelial function, in a cohort of young, low-risk adults.

Methods: One hundred sixty seven healthy subjects were included in the study. The effect of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, apolipoprotein (apo)-AI, apo-B and apo-E on endothelial function and inflammatory process was examined. Endothelial function was evaluated by determining forearm vasodilatory response to reactive hyperemia (RH%) using gauge-strain plethysmography. RH% was defined as the % change of forearm blood flow from baseline to the maximum flow during post-ischemic hyperemia. Endothelium independent dilatation in response to nitroglycerin (NTG%) was defined as the % change of forearm blood flow from baseline to the maximum flow after sublingual nitroglycerin administration.

Results: RH% was correlated with HDL ($r=0.267$, $p=0.001$), LDL ($r=0.355$, $p=0.0001$), triglycerides (rho=-0.366, $p=0.0001$), apo-AI ($r=0.240$, $p=0.004$) and apo-B ($r=-0.277$, $p=0.005$). NTG% was not affected by serum lipid levels. In multivariate linear regression, LDL ($\beta=-0.217$ [SE: 0.098], $p=0.028$), apo-AI ($\beta=0.277$ [SE: 0.124], $p=0.027$) and age ($\beta=0.916$ [SE:0.369], $p=0.015$) were independent predictors for RH% in this population (R^2 for the model: 0.243, $p=0.0001$).

Conclusions: Elevated lipid levels decrease forearm vasodilatory response to reactive hyperemia. Apolipoproteins, and especially apo-AI, are important determinants of endothelial function in these subjects, independently of LDL, HDL and triglycerides, implying that full measurement of the lipid profile may be of great importance in risk stratification of young individuals.

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There is evidence to suggest that hypercholesterolemia is a major independent risk factor for the development of atherosclerosis.[1] It has been shown previously that it leads to atherogenesis by increasing oxidative stress status[2] and by affecting endothelial function.[3] Moreover, the oxidation of low density lipoprotein (LDL) is a major step in atherogenesis, since it comprises the main mechanism for the formation of foam cells in the sub-endothelial space.[2] Although several studies have documented the impact of hypercholesterolemia on endothelial function,[4] the effect of each individual lipoprotein on endothelial function is still unclear.

In the present study we investigated the effect of apolipoproteins (apo) A1, B and E, in addition to the well-studied LDL, high density lipoprotein (HDL) and triglycerides, on forearm hyperemic response, an index of nitric oxide bioavailability in human forearm circulation, in a cohort of young low-risk subjects.

Methods

Population

The study population consisted of 167 conventionally healthy subjects of both sexes, as shown in Table 1. Subjects were recruited from among medical students, medical personnel and patients' relatives in our department. The participants had no risk factor for atherosclerosis (such as hypertension, smoking, diabetes mellitus, obesity or previously diagnosed hypercholesterolemia under treatment) and were receiving no medication. Subjects with elevated fasting cholesterol were first identified during this screening and remained in the study population, while only subjects with cholesterol >299 mg/dl were excluded.[5] Other exclusion criteria were use of any medication, antioxidant vitamin supplements or hormone replacement therapy during the past year, existence of any inflammatory disease, cancer, or laboratory evidence for liver or hematologic abnormalities. Patients with any heart or vascular disease (such as coronary artery disease or peripheral arterial disease) were also excluded. The protocol was approved by the Institutional Ethics Committee, and informed consent was given by each subject.

Forearm blood flow measurements

All measurements were performed between 10:00 and 13:00. The participants did not eat or drink any vasoactive agent, such as coffee or alcohol, for 12 hours before the study. Before measurements were started, subjects were rested in a supine position, in a dark, quiet room under a constant temperature of 22-25°C, for 30 minutes. Forearm blood flow was measured using gauge-strain plethysmography, as previously described.[6,7] Forearm blood flow was finally calculated as the % change of arm volume/100 ml tissue/minute.[6,8] The forearm vasodilatory response to reactive hyperemia (RH%) or to nitrates (NTG%) was defined as the percent change of flow from baseline to the maximum flow during post-ischemic hyperemia, or after sublingual administration of 0.8 mg nitroglycerin, respectively.[6,8]

Biochemical measurements

Venous blood samples were taken after a 12-hour fasting period and before plethysmography was performed. After centrifugation at 3500 rpm at 4° C for 15 minutes, plasma or serum was collected and stored at -80° C until assayed. Routine chemical methods (colorimetric enzymatic method in a Technicon automatic analyzer RA-1000, Date-Behring Marburg GmbH, Marburg, Germany) were used to determine serum lipid levels.

Statistical analysis

Analyses were performed using the SPSS 11.0 statistical package for Windows (SPSS Inc, Illinois, USA). Normally distributed data are presented as mean ± SEM, while those data not normally distributed were log-transformed for analysis and are presented as median (25th-75th percentile values). Comparisons between the two groups were performed using the unpaired t-test or Mann-Whitney-U test as appropriate. Correlations were assessed by Pearson's or Spearman's correlation method as appropriate.

Stepwise multivariate analysis was performed to

Table 1. Demographic characteristics and lipid profile of the participants.

	Total cholesterol <200 mg/dl	Total cholesterol ≥200 mg/dl
N (male/female)	114 (55/59)	53 (25/28)
Age (years)	30.9 ± 1.22	38.6 ± 2.12**
BMI (kg/m^2)	24.8 ± 2.2	25.1 ± 2.0
Cholesterol (mg/dl)	160.37 ± 2.08	235.85 ± 3.80**
Triglycerides (mg/dl) [a]	60.5 (45.0-84.0)	98.0 (72.0-145.0)**
HDL (mg/dl)	47.77 ± 1.18	44.10 ± 1.22*
LDL (mg/dl)	98.98 ± 2.11	165.76 ± 3.64**
Apo-A1 (mg/dl)	146.76 ± 2.74	137.43 ± 4.36
Apo-B (mg/dl)	73.78 ± 1.97	110.7 ± 4.17**
Apo-E (mg/dl)	3.55 ± 0.11	4.06 ± 0.16*

Apo – apolipoprotein; BMI – body mass index; HDL – high density lipoprotein; LDL – low density lipoprotein. Values expressed as mean ± SEM, except for [a]values expressed as median (25th-75th percentile values); *p<0.05 and **p<0.01 compared to subjects with total cholesterol <200 mg/dl.

Table 2. Endothelial function in young individuals: Stratification based on total cholesterol.

	Total cholesterol <200 mg/dl	Total cholesterol ≥200 mg/dl
Baseline forearm blood flow (ml/100 ml tissue/min)	4.95 ± 0.19	5.01 ± 2.22
Maximum hyperemic forearm blood flow (ml/100 ml tissue/min)	10.52 ± 0.67	8.03 ± 0.52*
Forearm vasodilatory response to reactive hyperemia (%)	102.07 ± 4.15	66.96 ± 5.12*
Maximum forearm blood flow after nitrate (ml/100 ml tissue/min)	7.72 ± 0.64	9.71 ± 1.11
Forearm vasodilatory response to nitrate (%)	65.25 ± 6.12	66.51 ± 4.87

Values expressed as mean ± SEM; *p<0.01 compared to subjects with total cholesterol <200 mg/dl

detect whether lipoproteins were independent predictors for RH%. Variables correlated with RH% at a 10% significance level were included into each of the multivariate models, while a backward elimination procedure was used to exclude variables from the models when significance did not reach the level of 5%. In all the analyses, two-tailed p values <0.05 were considered as statistically significant.

Results

Subjects with high total cholesterol levels (≥200 mg/dl) had significantly lower RH% (p<0.01, Table 2, Figure 1). In the overall population, RH% was correlated with HDL (r=0.267, p=0.001), LDL (r=-0.355, p=0.0001), triglycerides (rho=-0.366, p=0.0001), apo-A1 (r=0.240, p=0.004) and apo-B (r=-0.277, p=0.005) (Figure 2). Furthermore, RH% was also negatively correlated with age (r=-0.289, p=0.0001), while it was not correlated with Apo-E (r=-0.029, p=0.742).

In multivariate analysis, LDL (β=-0.217 [SE: 0.098], p=0.028), Apo-A1 (β=0.277 [SE: 0.124], p=0.027) and age (β=0.916 [SE: 0.369], p=0.015) were the only independent predictors for RH% in our population (R^2 for the model: 0.243, p=0.0001).

The apo-A1: apo-B ratio was also correlated with RH% in univariate analysis (r=0.303, p=0.0001). In a multivariate model where the apo-A1: apo-B ratio replaced apo-A1 and apo-B (R^2 for the model: 0.250, p=0.0001), apo-A1: apo-B ratio was not associated with RH% independently of LDL, HDL and triglyceride levels.

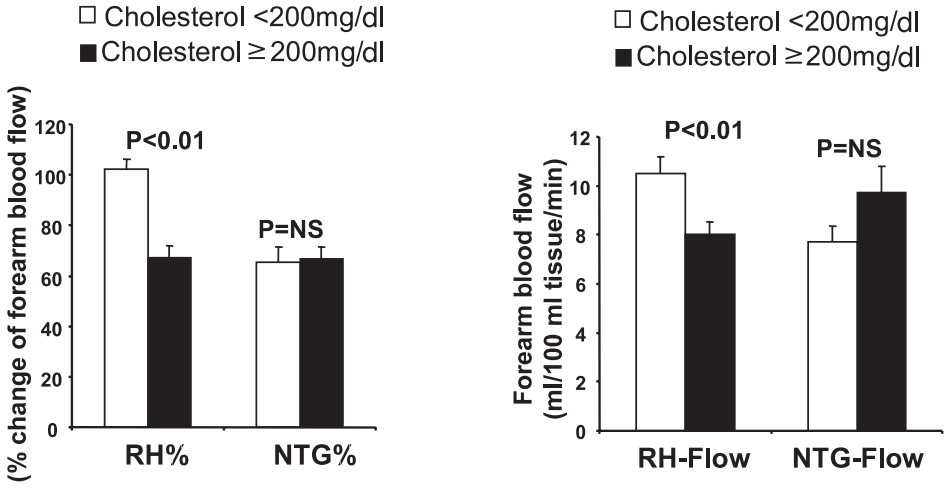

Figure 1. Subjects with fasting total cholesterol <200 mg/dl had significantly higher forearm vasodilatory response to reactive hyperemia (RH%), but not to nitrates (NTG%), compared to subjects with total cholesterol ≥200 mg/dl (left panel). Similarly, subjects with fasting total cholesterol <200 mg/dl had higher maximum hyperemic forearm blood flow (RH-Flow), but not maximum forearm blood flow, after nitrate administration (NTG-Flow) compared to subjects with total cholesterol ≥200 mg/dl (right panel). Values are presented as mean ± SEM.

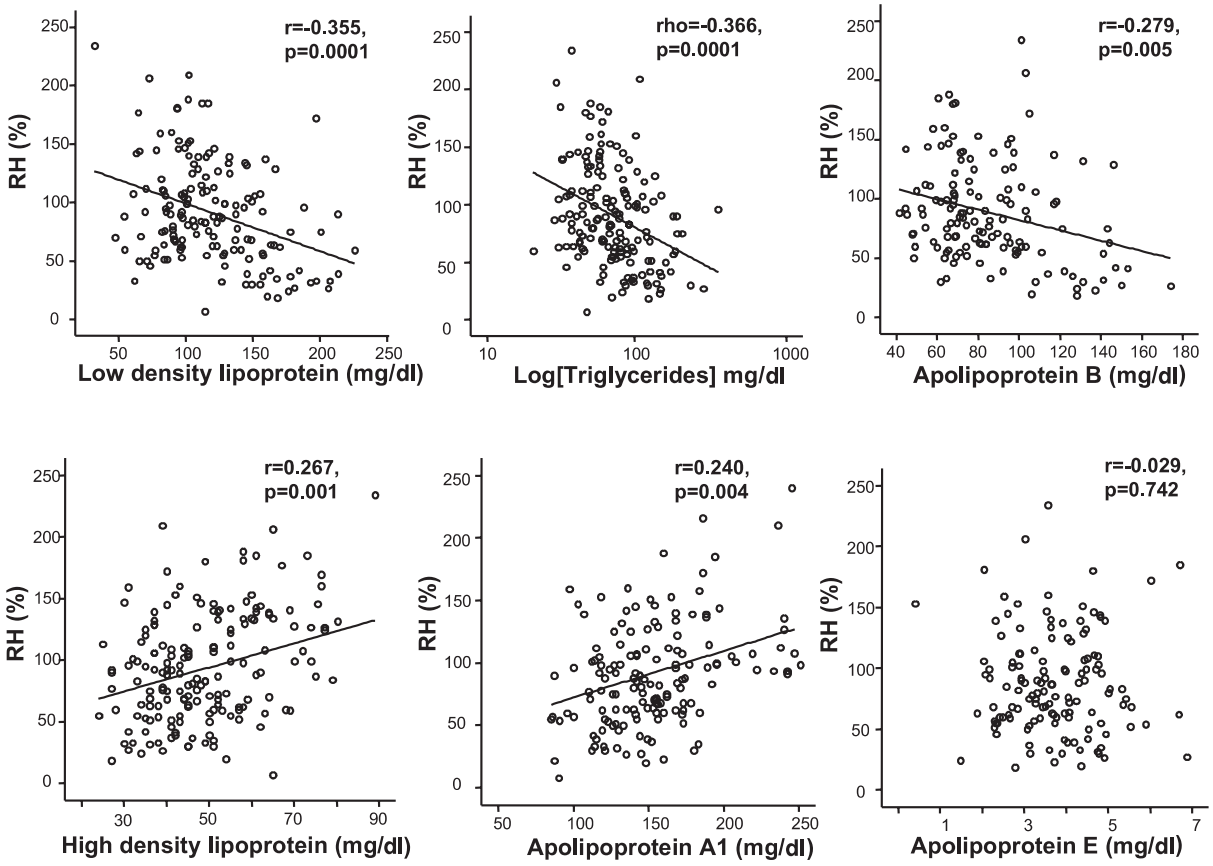

Figure 2. Correlations between lipoproteins and forearm vasodilatory response to reactive hyperemia (RH%).

NTG% was not correlated with any of the examined lipid levels, such as total cholesterol (r=0.067, p=0.635), triglycerides (rho=0.130, p=0.359), HDL (r=-0.066, p=0.651), apo-A1 (r=0.042, p=0.814), apo-B (r=0.085, p=0.633), and apo-E (r=0.092, p=0.603).

Discussion

The present study demonstrates that total cholesterol, triglycerides and apolipoproteins A1 and B are associated with a decreased forearm vasodilatory response to reactive hyperemia in relatively young and healthy individuals. We have also shown that LDL and apo-A1 are independent predictors for forearm vasodilatory response to reactive hyperemia, while they have no effect on endothelium-independent vasodilation. These findings imply that measurement of apo-A1 (in addition to the conventional measurements of cholesterol and triglycerides) may provide extra important information about endothelial function and the proatherogenic status in young individuals with no evidence of advanced atherosclerosis.

It has been reported previously that hypercholesterolemia leads to endothelial dysfunction through an increase in oxidative stress status.[9,10] Reactive oxygen species depress nitric oxide (NO) synthesis through their inhibitory effect on endothelial receptors for acetylcholine and other vasodilators,[2] while they react directly with NO to form peroxynitrite,[11] in this way decreasing NO bioavailability.[12] The oxidative modification of LDL in sub-endothelial space is a major step in atherogenesis, not only because it is the main mechanism leading to the formation of foam cells, but also because as a free radical, oxidized LDL is highly cytotoxic on vascular endothelium and it decreases NO bioavailability.[2] On the other hand, HDL has an opposite effect to LDL in atherogenesis, and acts directly on vascular endothelium, increasing NO synthesis.[26] These mechanistic data are compatible with the clinical observation that hypercholesterolemia leads to impaired endothelial function and decreased forearm hyperemic response in humans.[13,14]

Other lipid parameters are also associated with elevated cardiovascular risk, but it has been suggested

that LDL and triglycerides may not be the best discriminators for the presence of coronary artery disease.[15] Apolipoproteins are important components of lipoprotein particles, and there is accumulating evidence that measurement of various forms of apolipoprotein may improve the prediction of the risk of cardiovascular disease.[15-17]

Apolipoprotein B exists in two forms, apo-B-48 and apo-B-100. Apo-B-48 is synthesized in the intestine, where it is complexed with dietary triglycerides and free cholesterol absorbed from the gut lumen to form chylomicron particles. These are metabolized in the circulation and in the liver.[15] Apo-B-100 is synthesized in the liver and is present in LDL and VLDL particles. Apo-B is essential for the binding of LDL particles to the LDL receptor, allowing cells to internalize LDL. The total apo-B value indicates the total number of potentially atherogenic lipoproteins,[15] while excess of apo-B-containing particles is a main trigger in the atherogenic process.[15-17] Individuals with seemingly low or normal LDL levels can still be at increased risk of cardiovascular events,[15] and it has been noted that the level of apo-B and/or apo-B/apo-A1 in the plasma may be a better predictor.[16,17] However, despite the prognostic role of apo-B in cardiovascular risk, the underlining mechanisms of its action have not been thoroughly investigated. Although it seems that it is correlated with carotid intima/media thickness,[18,19] its effect on nitric oxide bioavailability and endothelial function in general is obscure.

Apo-A1 acts as a cofactor for lecithin cholesterol acyl transferase (LCAT), which is important in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport to the liver.[20] Furthermore, Apo-A1 is the ligand for the ATP-binding cassette (ABC) protein, and hence is involved in the docking procedure by which excess cholesterol in peripheral cells is externalized to HDL for further reverse cholesterol transport, either directly or indirectly via LDL, back to the liver.[21] Levels of apo-A1 are strongly correlated with those of HDL, and expression of apo-A1 may be largely responsible for determining the plasma level of HDL.[22] At a clinical level, apo-A1 is a strong predictor for cardiovascular disease,[23] but it is unclear whether it affects vascular function independently from HDL and other lipids. Indeed, although HDL has a number of well established beneficial effects on vascular endothelium and increases nitric oxide bioavailability, the direct role of apo-A1 is obscure.[24] Recent evidence suggests that apo-A1 increases the amount of endothelial nitric oxide synthase (eNOS) protein in cultured human vascular endothelial cells,[25] increasing nitric oxide synthesis,[26] but its role *in vivo* remains unclear.

In the present study we examined whether apolipoproteins A1 and B have a direct impact on endothelial function in conventionally healthy young individuals, with no risk factors for atherosclerosis, independently from LDL and HDL levels. We have shown that forearm hyperemic response is decreased in subjects with hypercholesterolemia. This marker of endothelial function was correlated with HDL and apo-A1, while it was negatively affected by LDL, triglycerides, and apo-B. Interestingly, only LDL and apo-A1 were independent predictors for endothelial function in these subjects. This finding implies that apo-A1 may be directly implicated in the development of endothelial dysfunction, independently from HDL. Further studies are needed to evaluate the molecular mechanisms of this effect.

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