Early Vascular Damage in Primary Hypoalphalipoproteinemia

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The relationship between hypoalphalipoproteinemia (hypoα), a metabolic disorder characterized by reduced high-density lipoprotein (HDL) cholesterol levels, and atherosclerotic disease is not completely understood. We investigated arterial functional and structural changes in 19 subjects with hypoα (HDL cholesterol $\leq$ 0.7 mmol/L for men and $\leq$ 0.8 mmol/L for women; 13 men; 47 $\pm$ 7 years) and in 21 healthy control subjects (11 men; 46 $\pm$ 13 years). Brachial-artery flow-mediated vasodilation (FMV) and intima-media thickness (IMT) of the carotid and femoral arteries were determined in all subjects. FMV was significantly lower in hypoα than in controls (5.6% $\pm$ 4.3% *v* 8.2% $\pm$ 2.7%; $P < .05$). IMT was greater in hypoα than in controls at both the internal carotid (0.83 $\pm$ 0.1 mm *v* 0.69 $\pm$ 0.1 mm) and superficial femoral level (0.83 $\pm$ 0.2 mm *v* 0.68 $\pm$ 0.1 mm; both $P < .05$). FMV had a positive correlation with HDL cholesterol ($r = .42$, $P = .06$) and a negative one with triglycerides ($r = -0.38$, $P = .01$). An inverse relationship was found between HDL cholesterol and internal carotid and superficial femoral IMT ($r = -0.64$ and $r = -0.60$, respectively; $P < .01$ for both) and a positive one between triglycerides and internal carotid and superficial femoral IMT ($r = .53$ and $r = .47$, $P < .05$). In a multivariate regression analysis, brachial FMV was predicted by HDL cholesterol and brachial diameter ($\beta = .42$ and -0.43, respectively; both $P < .05$). HDL cholesterol was the only significant predictor of internal carotid and superficial femoral IMT ($\beta = -0.45$ and -0.49, respectively; both $P < .05$). In conclusion, subjects with primary hypoα, without overt cardiovascular disease, are characterized by an impaired endothelial function and by an increase in large-artery IMT.

PRIMARY hypoalphalipoproteinemia (hypoα) is a relatively rare metabolic disorder characterized by low high-density lipoprotein (HDL) cholesterol levels caused by genetic defects with a dominant or nondominant transmission.[1] Several epidemiologic, clinical, and intervention studies have shown that a low HDL cholesterol level is a risk factor for cardiovascular disease.[2-6] However, the association between primary hypoα and atherosclerosis is still controversial.[1] Indeed, due to its genetic heterogeneity, patients with hypoα do not show a uniformly increased risk for cardiovascular disease.[1] Moreover, the limited number of individuals with very low HDL cholesterol level and the frequent association of the hypoα trait with other cardiovascular risk factors make previous observations not fully descriptive of the real association between this metabolic condition and atherosclerotic disease.[1]

Endothelial dysfunction and wall thickening of the large arteries represent the earliest evidence of vessel atherosclerotic involvement. Brachial flow-mediated vasodilation (FMV) is a noninvasive measure of endothelium-dependent vasoactivity mediated by nitric oxide,[7] and it also represents an indirect estimate of coronary artery endothelial function.[8] Carotid intima-media thickness (IMT) is considered a reliable noninvasive estimate of the early atherosclerotic burden[9] and an independent predictor of cardiovascular events.[10]

We hypothesized that brachial FMV and carotid and femoral IMT might be useful to investigate the onset of early vascular damage in adult subjects with severe primary hypoα defined for HDL levels below the 5th percentile of the Italian population.[11]

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MATERIALS AND METHODS

We examined 19 patients (13 men; mean age, 47 $\pm$ 7 years) who had been referred to our lipid clinic with a diagnosis of primary hypoα. Inclusion criteria were HDL cholesterol levels below the 5th percentile of the Italian population ($\leq$0.7 mmol/L for men, $\leq$ 0.8 mmol/L for women) determined in $\geq$ 3 determinations within 3 months.[11] We included 13 patients with triglyceride levels $<$ 2.2 mmol/L and 6 patients with plasma triglyceride between 2.2 mmol/L and 4.5 mmol/L.

We excluded all conditions able to affect substantially HDL cholesterol levels, including obesity (body mass index [BMI] $>$ 30 kg/m^2), diabetes, liver or connective tissue disease, cancer, infection in the previous 3 months, smoking, hormone replacement therapy, or hypolipemic therapy. For their influence on brachial FMV,[12] we also excluded hypertensive patients and subjects under antiplatelet and antioxidant treatment. The presence of familial hypoα was demonstrated in 13 subjects by the existence of vertical transmission of the trait through 2 generations. History and clinical examination were negative for previous cardiovascular disease, including myocardial infarction, angina, peripheral vascular disease, transient cerebral ischemia, and stroke. We also examined a control group of 21 subjects (11 males; mean age, 46 $\pm$ 13 years), normolipemic, with HDL levels over the 5th percentile of Italian population without diabetes, hypertension, smoking habits, previous cardiovascular events, and not using drugs acting on endothelial function. All subjects underwent clinical examination, measurement of brachial-artery FMV, carotid and femoral IMT, and serum lipids. Blood was drawn in the morning after 13-hour fasting. The following parameters were determined: total cholesterol, triglycerides (enzymatic colorimetric method), HDL cholesterol (enzymatic colorimetric method after precipitation to polyethilen-glycole), HDL_2 cholesterol and HDL_3 cholesterol subfractions (polyethylene-glycole Immuno AG, Vienna, Austria), and low-density lipoprotein (LDL) cholesterol (Friedewald formula).[13]

Brachial Artery Ultrasound

FMV was assessed on the brachial artery by ultrasonography. Details of the procedure, which was performed according to the International Brachial Artery Task Force guidelines,[14] have been reported else-

Table 1. Characteristics of Patients With Hypoα and Healthy Control Subjects

	Hypoα (n = 19)	Controls (n = 21)
Age (yr)	47 ± 7	46 ± 13
Male/female	13/6	11/10
Body mass index (kg/m^2)	26.2 ± 1.9*	23.2 ± 2.8
Systolic blood pressure (mm Hg)	131 ± 8	122 ± 12
Diastolic blood pressure (mm Hg)	75 ± 5	75 ± 6
Total cholesterol (mmol/L)	5.5 ± 0.8	5.1 ± 1.1
LDL cholesterol (mmol/L)	3.3 ± 0.9	3.3 ± 1.0
HDL cholesterol (mmol/L)	0.7 ± 0.1*	1.3 ± 0.3
HDL_2 cholesterol (mmol/L)	0.3 ± 0.2*	0.6 ± 0.2
HDL_3 cholesterol (mmol/L)	0.4 ± 0.2*	0.7 ± 0.2
Triglycerides (mmol/L)	3.1 ± 1.4*	1.2 ± 0.6

*$P < .05$ *v* controls.

where.[15] The measurements were performed in supine position on the nondominant arm after 10 to 20 minutes resting in a quiet, dark room with a temperature of 22°C. The brachial artery was scanned longitudinally just above the antecubital crease using a 10-MHz probe (ESAOTE AU4, Florence, Italy). Diameter of the brachial artery was measured at the R wave of the electrocardiogram, on the interface between media and adventitia of the anterior and posterior wall. Gain settings were optimized to identify the lumen and the vessel wall interfaces and were not modified during the examination. Hyperemia was induced by inflation of a pneumatic cuff (12.5 cm wide) at 230 to 250 mm Hg for 4 minutes on the most proximal portion of the upper arm. Arterial diameter measurement was repeated 45 to 60 seconds after sudden deflation of the cuff. Tracings were recorded on videotape and read by one investigator, who was unaware of the subject's clinical data and temporal sequence. The average of 3 measurements of basal and posthyperemia diameter was used for the analysis. FMV was expressed as the relative increase in brachial artery diameter during hyperemia and defined as $100 \times$ [(posthyperemia diameter − basal diameter)/basal diameter]. Blood flow was measured as arterial cross sectional area ($\pi \times r^2$) times mean Doppler velocity corrected for angle. The intraobserver between-occasion reproducibility of FMV in our laboratory was assessed in 10 subjects examined 2 days apart. The mean ± SD difference between the 2 examinations was 1.0% ± 1.5%.

Carotid and Femoral IMT

Carotid and femoral arteries were examined with high-resolution B-mode ultrasonography.[16,17] The examination was performed with a commercially available ultrasound device (HDI 3500; Advanced Technology Laboratories, Bothell, WA) equipped with a linear multifrequency 5 to 12 MHz transducer. Subjects were examined in the supine position, and all measurements were obtained at end-diastole by electrocardiographic triggering. The ultrasound images have been stored on a S-VHS videotape and analyzed using an image processing workstation (Kontron KS-200, Munich, Germany). On a longitudinal 2-dimensional ultrasound image of the carotid and femoral artery, the near and far arterial walls are displayed as 2 bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line on the far wall (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) indicates the IMT of the far wall. For the near wall, IMT was calculated as the distance between the trailing edge of the first bright line and the trailing edge of the second bright line. A 1.5-cm segment of the common carotid artery (immediately caudal to the bifurcation), the bifurcation of the common carotid artery, and the proximal 1.5-cm segment of the internal carotid artery were considered. Similarly, we examined the distal 1.5-cm segment of the common femoral artery and the proximal 1.5-cm segment of the superficial femoral artery. Tracings were read by 2 observers who were unaware of patients' clinical data. Each subject was characterized by: mean carotid IMT (defined as the average of 36 IMT readings: common, bifurcation and internal carotid arteries, right and left side, far and near wall, 3 sampling points per segment), mean femoral IMT (defined as the average of 24 IMT readings: common and superficial femoral arteries, right and left side, far and near wall, 3 sampling points per segment), mean-maximum carotid IMT (defined as the average of all maximum IMT for each segment - as many as 12 readings: common, bifurcation and internal carotid arteries, right and left side, far and near wall, 3 sampling points per segment), and mean-maximum femoral IMT (defined as the average of all maximum IMT for each segment - as many as 8 readings: common and superficial femoral arteries, right and left side, far and near wall, 3 sampling points per segment). We also calculated the mean and the mean-maximum IMT of each segment, including common carotid, bifurcation, internal carotid, common femoral, and superficial femoral arteries. The intraobserver coefficient of variation was 3.9% in the carotid district (mean ± SD of the difference 0.018 ± 0.031 mm) and 5.2% in the femoral district (mean ± SD of the difference 0.006 ± 0.028 mm). Corresponding interobserver values were 5.6% in the carotid district (0.028 ± 0.032 mm) and 7.7% in the femoral district (0.029 ± 0.034 mm).

Statistical Analysis

Data are presented as mean ± SD. Student's *t* test was performed to compare parametric variables between cases and controls. Pearson's simple and partial correlation coefficients tested univariate association between study variables. Multiple regression analysis was performed with mean internal carotid IMT, mean superficial femoral IMT, and brachial FMV as dependent variables. The independent variables in the model were age, sex, triglycerides, HDL cholesterol, LDL cholesterol, BMI, and systolic and diastolic blood pressure. The FMV model also included brachial-artery diameter. *P* levels $< .05$ were considered statistically significant. Data were stored by SPSS statistical package, release 10.0 (SPSS, Chicago, IL).

RESULTS

Demographic, clinical, and lipid parameters of the 40 study subjects are reported in Table 1. As expected, patients had lower values of HDL cholesterol (0.7 ±0.1 *v* 1.3 ± 0.3 mmol/L), HDL_2 cholesterol (0.3 ± 0.2 *v* 0.6 ± 0.2 mmol/L), HDL_3 cholesterol

Table 2. Ultrasound Characteristics of Study Subjects

	Hypoα (n = 19)	Controls (n = 21)
Common carotid mean IMT (mm)	0.75 ± 0.1	0.73 ± 0.1
Bifurcation mean IMT (mm)	0.78 ± 0.1	0.72 ± 0.1
Internal carotid mean IMT (mm)	0.83 ± 0.1*	0.69 ± 0.1
Carotid max IMT (mm)	0.80 ± 0.2	0.73 ± 0.1
Carotid mean IMT (mm)	0.79 ± 0.1	0.72 ± 0.1
Common femoral mean IMT (mm)	0.75 ± 0.1	0.73 ± 0.1
Superficial femoral mean IMT (mm)	0.83 ± 0.2*	0.68 ± 0.1
Femoral max IMT (mm)	0.78 ± 0.2	0.74 ± 0.1
Femoral mean IMT (mm)	0.75 ± 0.1	0.70 ± 0.1
FMV (%)	5.6 ± 4.3*	8.2 ± 2.7
Brachial-artery diameter (mm)	4.2 ± 0.7	3.8 ± 0.7
Basal brachial flow (mL/min)	98 ± 21	101 ± 6
Post-hyperemic flow (mL/min)	234 ± 32	220 ± 17

Abbreviations: IMT, intima-media thickness; FMV, flow-mediated vasodilation.

*$P < .05$ *v* controls.

Table 3. Univariate Correlations Between Internal Carotid Mean IMT, Superficial Femoral Mean IMT, Brachial Flow-Mediated Vasodilation, and Lipid Parameters

	Brachial Flow-Mediated Vasodilation		Internal Carotid Mean IMT		Superficial Femoral Mean IMT	
	r	*P*	*r*	*P*	*r*	*P*
LDL cholesterol	.13	.40	−.12	.45	−.16	.31
HDL cholesterol	.42	.006	−.64	.001	−.60	.001
HDL_2 cholesterol	.39	.01	−.52	.001	−.41	.009
HDL_3 cholesterol	.34	.02	−.53	.001	−.41	.007
Triglycerides	−.38	.01	.53	.001	.47	.002

(0.4 ± 0.2 *v* 0.7 ± 0.2 mmol/L), and higher values of triglycerides (3.1 ± 1.4 *v* 1.2 ± 0.6 mmol/L) than control subjects. In addition, subjects with hypoα had higher BMI. Ultrasound measurements are reported in Table 2. Patients had lower values of FMV (5.6 ± 4.3 *v* 8.2% ± 2.7%) and a higher mean IMT at both the internal carotid (0.83 ± 0.1 *v* 0.69 ± 0.1 mm) and superficial femoral (0.83 ± 0.2 *vs* 0.68 ± 0.1 mm) with respect to controls.

Correlates of Ultrasound Measurements

At the univariate analysis as shown in Table 3, FMV had a positive correlation with HDL cholesterol ($r = .42$, $P = .006$), HDL_2 and HDL_3 subfractions ($r = .39$, $P = .01$ and $r = .34$, $P = .02$) and a negative one with triglycerides ($r = -0.38$, $P = .01$). Internal carotid mean IMT was related directly to triglycerides ($r = .53$, $P = .001$), inversely with HDL cholesterol ($r = -.64$, $P = .001$), and HDL_2 and HDL_3 subfractions ($r = -.52$, $P = .001$ and $r = -.53$, $P = .001$). Superficial femoral mean IMT had a direct association with triglycerides ($r = .47$, $P = .002$) and an inverse one with HDL cholesterol ($r = -.60$, $P = .001$) and HDL_2 and HDL_3 subfractions ($r = -.41$, $P = .009$ and $r = -.41$, $P = .007$). Multivariate regression analysis showed that HDL cholesterol and brachial-artery diameter were both predictive of brachial FMV ($\beta = 0.42$ and -0.43, respectively; both $P < .05$, see Table 4), and HDL cholesterol was the only independent predictor of internal carotid and superficial femoral mean IMT ($\beta = -0.45$ and -0.49, respectively; both $P < .05$, see Table 5).

DISCUSSION

Our study showed that subjects with severe primary hypoα and no clinical overt atherosclerotic disease have subclinical evidence of early arterial structural and functional damage, including impaired endothelial function and increased IMT at both the carotid and femoral level.

In the present study, brachial-artery FMV, which is considered a reliable marker of arterial endothelial function, was lower by about one third in subjects with hypoα than those with normal HDL cholesterol levels. Noteworthy, HDL cholesterol levels were the only independent predictor of FMV together with brachial artery diameter. In agreement with our findings, previous experimental evidence has shown a positive effect of HDL on the endothelial function. In vitro, HDL promotes nitric oxide synthesis through a direct stimulation of endothelial nitric oxide synthase,[18] promotes prostacyclin synthesis, and inhibits eicosanoid production.[19,20] In addition, HDL reduces oxidized LDL generation,[21] limits the inhibitory effects of oxidized LDL on the endothelial nitric oxide synthase activity,[22] and reverses endothelial damage induced by oxidized LDL.[23] These latter mechanisms might have important pathophysiologic implications, because oxidized LDL is an important trigger of early atherosclerosis.[24] Indeed, HDL are carriers of key antioxidant enzymes and proteins, including platelet-activating factor acetylhydrolase, paraoxonase, and apoprotein J, and are thus able to protect endothelium from oxidative

Table 4. Predictors of Brachial Flow-Mediated Vasodilation

Variable	β	*P*
Age	−0.10	.53
Sex	−0.001	.99
Body mass index	0.10	.52
Systolic blood pressure	−0.18	.27
Diastolic blood pressure	−0.06	.73
LDL cholesterol	0.05	.75
HDL cholesterol	0.42	.04
Triglycerides	−0.07	.73
Brachial diameter	−0.43	.008

NOTE. Significance of the model $P = .022$.

Table 5. Predictors of Internal Carotid Mean IMT and Superficial Femoral Mean IMT

	β	*P*
Internal carotid mean IMT		
Age	−0.09	.54
Sex	0.04	.75
Body mass index	0.07	.60
Systolic blood pressure	0.08	.58
Diastolic blood pressure	0.10	.51
LDL cholesterol	−0.05	.74
HDL cholesterol	−0.45	.02
Triglycerides	0.22	.23
Significance of the model $P = .003$		
Superficial femoral mean IMT		
Age	−0.04	.78
Sex	0.16	.29
Body mass index	0.20	.19
Systolic blood pressure	−0.13	.39
Diastolic blood pressure	0.05	.73
LDL cholesterol	−0.16	.33
HDL cholesterol	−0.49	.01
Triglycerides	0.08	.67
Significance of the model $P = .005$.		

stress.[25-27] The effects of HDL cholesterol on the endothelial function have been investigated also in humans.[28-30] HDL cholesterol has been associated with coronary endothelial reactivity in old subjects with established cardiovascular disease[28] and is able to affect endothelial function in healthy subjects.[29,30] More recently, Spieker et al[31] found that in hypercholesterolemic patients intravenous infusion of reconstituted HDL rapidly normalizes endothelium-dependent vasodilation by increasing nitric oxide bioavailability. However, to the best of our knowledge, this is the first study in which the impairment of endothelial function in subjects with severe primary hypoα was observed.

We also found that subjects with severe primary hypoα have increased IMT at the internal carotid and superficial femoral artery, HDL cholesterol being the only independent predictor of IMT. Several studies demonstrated that low HDL cholesterol is associated with a thickening of the carotid wall in patients with known coronary artery disease,[33] in middle-age subjects free from cardiovascular events,[34] and in hyperlipemic patients.[35,36] More recently, Baldassarre et al[35] described an increased carotid IMT in subjects with hypoα. Our study differed in that we included only subjects free from overt cardiovascular disease.

The impact of triglycerides and visceral adiposity on endothelial function and arterial IMT has been investigated extensively[32,34,37-39] Our previous study has shown an association between serum triglyceride levels and endothelial dysfunction in hypertriglyceridemic patients.[40] Previous studies demonstrated an altered arterial FMV and an increased carotid IMT in subjects with body fat accumulation.[38,39] In this sense, the higher BMI and triglyceride levels in Hypoα than in controls may have had an influence on the different FMV and IMT observed in the study groups. However, we can exclude that BMI and triglycerides influenced to a significant extent the relationship between HDL cholesterol, brachial FMV, and arterial IMT, because multivariate analysis, including triglycerides and BMI as potential confounders, clearly shows an inverse independent association between HDL cholesterol concentration and arterial structural and functional changes.

In conclusion, the present study demonstrated for the first time the presence of functional and structural changes in the vascular wall of subjects with primary hypoα without present or past signs or symptoms of cardiovascular disease.

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