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Chapter 3

Patients with low HDL-cholesterol caused by mutations in LCAT have increased arterial stiffness

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Submitted
ABSTRACT

Carriers of a functional mutation in LCAT, encoding lecithin:cholesterol acyl transferase, are exposed to lifelong low high-density lipoprotein cholesterol levels. Whether LCAT mutation carriers have an increased risk of cardiovascular disease is under dispute. Since previous ultrasound studies of atherosclerosis were inconclusive, we investigated functional alterations in large arteries by measuring arterial stiffness by carotid-femoral pulse wave velocity. We hypothesized that arterial stiffness is increased in LCAT mutation carriers and is associated with carotid wall thickening. We assessed 45 carriers of LCAT mutations (mean age±SD 46±13 yrs) and 45 age-matched controls. Probands referred with established cardiovascular disease were excluded. In carriers, high-density lipoprotein cholesterol was lower (32±12 vs 59±16 mg/dl; \( p < 0.0001 \)) and triglycerides higher (median 116 [IQR 80-170] vs 71 [IQR 53-89] mg/dl; \( p < 0.001 \)) vs. controls. Pulse wave velocity was higher in carriers vs. controls (7.9±2.0 m/s vs 7.1±1.6 m/s; \( p < 0.01 \)). This difference retained significance in multivariate analysis after adjustment for age, sex, mean arterial pressure and body mass index, and after exclusion of carriers and controls with cardiovascular disease. Both in carriers and controls, pulse wave velocity was correlated with wall thickening of the carotid arteries as assessed by ultrasound (\( R=0.50, p<0.001 \) for carriers and \( R=0.36, p<0.04 \) for controls) and 3.0 Tesla magnetic resonance imaging (\( R=0.54, p<0.001 \) for carriers and \( R=0.58, p<0.001 \) for controls). In conclusion, pulse wave velocity is increased in LCAT mutation carriers with high density lipoprotein cholesterol and is associated with carotid wall thickening.
INTRODUCTION

A low plasma level of high-density lipoprotein cholesterol (HDL-c) is a strong and independent predictor of cardiovascular disease (CVD).1, 2 Carriers of mutations in LCAT, encoding lecithin:cholesterol acyl transferase, are exposed to lifelong low HDL-c levels. LCAT is a crucial enzyme in HDL-c metabolism produced in the liver and small intestine.3, 4 Upon secretion into the circulation, it associates predominantly with HDL where it esterifies free cholesterol using apolipoprotein A-I (apoA-I) as a cofactor4. Carriers of LCAT mutations have been reported to suffer from increased atherosclerosis, but this is disputed. In this respect, two ultrasound studies reached opposite conclusions, one study reporting increased intima media thickness (cIMT) in the carotid arteries of carriers as a surrogate outcome for atherosclerosis5 and the other reporting decreased IMT in carotid arteries of carriers compared controls.6 We have recently confirmed the findings of our earlier study by showing structural abnormalities in the carotid arteries of LCAT mutation carriers with low HDL-c compared to matched controls using both ultrasound and 3.0 Tesla magnetic resonance imaging of the carotid arteries (Duivenvoorden et al, JACC accepted 2011). Whether the observed atherosclerotic structural changes in LCAT mutation carriers are associated with functional alterations of large arteries is undetermined. Arterial stiffness is a strong and independent predictor of CVD.7-10 Pulse wave velocity (PWV), the gold standard of non-invasive measurement of arterial stiffness,11 has emerged as a novel biomarker for predicting cardiovascular mortality and morbidity.

We hypothesized that patients with LCAT gene mutations, with lifelong exposure to low HDL-c levels, would have increased arterial stiffness compared to healthy controls. We therefore studied carotid-femoral PWV, in patients with LCAT gene mutations and age-matched controls. In addition to our primary study objective, we also examined the association between arterial stiffness and structural changes in large arteries by assessment of wall thickening of the carotid arteries using B-mode ultrasound (cIMT) and 3.0 Tesla magnetic resonance imaging (MRI).

METHODS

Study design and participants
The design of this study has been described in detail (Duivenvoorden et al, JACC accepted 2011). In brief, the study was conducted at the Academic Medical Center in Amsterdam, The Netherlands from October 2008 to October 2009. The study protocol was approved by the local institutional review board and all subjects provided written informed consent. Patients molecularly diagnosed with LCAT mutations were enrolled in this study, irrespective of their age and sex. In order to limit referral bias, we excluded family probands who were referred to our outpatient clinic with clinically manifest CVD. For the control group, unaffected family members of the included carriers were asked to participate in the study, comprising first, second or third
degree family members or spouses. These controls were included if they could be individually matched for age to carriers. Because the number of family controls was insufficient the control group was complemented with unrelated controls recruited by advertisement. Family history of CVD, presence of cardiovascular risk factors, use of medication and alcohol were assessed. Presence of hypertension was defined as a systolic blood pressure (SBP) $>$ 140 mmHg, a diastolic blood pressure (DBP) $>$ 90 mmHg or use of antihypertensive medication.

**Blood pressure and arterial stiffness**

Participants visited the hospital after an overnight fast and were asked to refrain from smoking (if applicable) at least three hours before the visit. All measurements were carried out in supine position after 15 minutes rest in a quiet, temperature-controlled room. All hemodynamic measurements were performed by a single investigator (BvdB) who was blinded for the genetic status of the participants. Brachial blood pressure was measured 3 times at 1-minute intervals in supine position at the right arm after 15 minutes rest using a validated oscillometric device (Omron 705IT). The mean of the last 2 measurements was used for analysis. Measurements of carotid-femoral pulse wave velocity (PWV) were performed with the SphygmoCor system (Atcor Medical Pty Ltd, West Ryde, Australia). Pulse waveforms were recorded at the right carotid and femoral artery sequentially. Wave travel distance was calculated by subtracting carotid artery – suprasternal notch distance from suprasternal notch – femoral artery distance. Measurements were conducted in duplicate and means were used for analysis.

**Carotid ultrasound and magnetic resonance imaging**

Ultrasound scans of the carotid arterial wall were assessed as surrogate outcome for atherosclerosis according to a standardized protocol.\(^{12}\) Values given are means of left and right common carotid artery, carotid bulb and internal carotid. Bilateral 3.0 Tesla (T) MRI scans of the carotid arteries were obtained as previously described (Duivenvoorden et al, JACC accepted 2011). Normalized wall index (NWI) represents mean vessel wall area normalized for the transverse size of the vessel, measured as the outer wall area. Carotid IMT and NWI were assessed during the same hospital visit as PWV.

**Plasma lipids**

Blood was obtained after overnight fasting and stored using standardized protocols. Plasma total cholesterol, HDL-c and triglyceride levels were analyzed using a commercially available enzymatic method (Westburg, USA) on a Cobas Mira autoanalyzer (Roche, Switzerland). Low density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald equation.

**Statistical analysis**

Data are expressed as means±standard deviations (SD), median (interquartile range [IQR]) or numbers and percentages where appropriate. Differences between carriers and controls were
assessed by comparison of continuous data using independent t-tests for parametric data and Mann-Whitney for non-parametric data; chi-square test was applied to compare categorical data. Correlations are expressed as Pearson’s correlation coefficient (R). A multivariate model was used with generalized estimating equations in the SAS procedure GENMOD to account for potential confounders, i.e. age, gender, mean arterial pressure (MAP) and body mass index (BMI) and correlations within families due to clustering of genetic and/or environmental factors, using stepwise backward elimination. Statistical analyses were done using SPSS (Statistical Package for the Social Sciences) version 16.0 and SAS package version 9.1 (SAS Institute Inc., Cary, NC USA). The authors had full access to the raw data and take responsibility for its integrity.

RESULTS

Population characteristics

We studied 45 carriers of LCAT gene mutations from 15 families, all of Dutch descent, and 45 age-matched controls of which 19 were family members and 26 were unrelated individuals. Of the carriers, 43 had one mutant LCAT allele, while 1 was homozygous for T147I and one was compound heterozygous for T147I and V333M. Both the homozygote and the compound heterozygote had previously presented with corneal opacification and HDL deficiency, without the presence of proteinuria, and had thus been diagnosed with fish eye disease (FED).

| Table 1 Clinical characteristics of LCAT mutation carriers compared to unaffected controls |
|---------------------------------|-------------------------------|------------------|----------|
| n                              | **LCAT mutation carriers**    | **Matched controls** | **p-value** |
| Age, years                     | 46 ± 13                       | 45 ± 14           | 0.83     |
| Male sex, n (%)                | 35 (78%)                      | 29 (64%)          | 0.16     |
| BMI, kg/m²                     | 26.3±4.1                      | 24.6±3.0          | 0.02     |
| Smoking, n (%)                 | 7 (16%)                       | 5 (11%)           | 0.54     |
| History of CVD, n (%)          | 6 (13%)                       | 1 (2%)            | 0.05     |
| Hypertension, n (%)            | 17 (38%)                      | 13 (29%)          | 0.37     |
| SBP, mmHg                      | 135 ± 15                      | 131 ± 13          | 0.12     |
| DBP, mmHg                      | 79 ± 9                        | 77 ± 9            | 0.19     |
| Statin users, n (%)            | 16 (36%)                      | 1 (2%)            | <0.001   |
| Total Cholesterol, mg/dl       | 172 ± 47                      | 191 ± 35          | 0.05     |
| LDL-c, mg/dl                   | 125 ± 35                      | 125 ± 35          | 0.75     |
| HDL-c, mg/dl                   | 32 ± 12                       | 59 ± 16           | <0.001   |
| Triglycerides, mg/dl           | 116 [IQR 80-170]              | 71 [IQR 53-89]    | <0.001   |

Values are indicated as means ± SD or median [IQR] unless otherwise indicated.
Table 1 summarizes the demographic, lifestyle, and clinical characteristics of carriers and controls. Proper matching for age was achieved for carriers of an LCAT mutation and controls. The percentage of males did not differ between the two groups. On average, carriers had a 1.9 kg/m² higher BMI (p=0.02). More carriers had experienced cardiovascular events and received statin treatment than controls, and carriers tended to receive antihypertensive treatment more frequently than controls. Mean blood pressure was 135±15/79±9 mmHg for LCAT mutation carriers and 131±13/77±9 mmHg for controls (p=0.12/p=0.19). HDL-c levels in carriers of LCAT mutations were lower compared to controls (32±12 vs 59±16 mg/dl, p<0.001), while LDL-c levels were identical (125±35 mg/dl for LCAT carriers and 125±31 mg/dl for controls, p=0.65). Triglycerides were higher in carriers of LCAT mutations: 116 [IQR 80-170] mg/dl compared to 71 [IQR 53-89] mg/dl in controls (p<0.001).

Pulse wave velocity in carriers of LCAT mutations and age-matched controls

PWV was higher in carriers of a mutation in LCAT compared to controls, 7.9±2.0 vs 7.1±1.6 m/s (p<0.01), see Figure 1. In a multivariate regression model that adjusted for age, sex, MAP, BMI

![Figure 1 Carotid-femoral pulse wave velocity in carriers of an LCAT mutation and matched controls](image)

Boxed values are means ± SD for PWV. *Difference retained significance in multivariate analysis, independent of age, sex, mean arterial pressure and BMI and correlations within families due to clustering of genetic and/or environmental factors, and also after exclusion of 6 matched pairs with members with a history of CVD.
Arterial stiffness in LCAT deficiency

and family clustering, this difference retained statistical significance ($p<0.01$). After exclusion of matched pairs of which the carrier ($n=6$) and/or control ($n=1$) had suffered from CVD, the PWV of the 38 remaining carriers remained significantly higher compared to the respective matched controls ($7.7\pm2.0$ and $6.9\pm1.6$ m/s, $p<0.05$). Again, this difference retained significance after adjustment for age, sex, MAP and family clustering in the multivariate regression model ($p<0.01$). PWV was not correlated with HDL-c in LCAT mutation carriers ($R=-0.05$, $p=0.75$) or controls ($R=0.13$, $p=0.41$).

**PWV and carotid wall thickening assessed by ultrasound and 3.0 T MRI**

In a random set of 36 carriers and 36 controls individually matched for age, carotid wall thickening was assessed by ultrasound (cIMT) and by 3.0 T MRI scanning (Duivenvoorden et al, JACC

**Table 2** Correlations of Carotid-Femoral Pulse Wave Velocity with Normalized Wall Index (MRI) and Carotid Intima Media Thickness (Ultrasound) in LCAT mutation carriers and controls

<table>
<thead>
<tr>
<th></th>
<th>Normalized Wall Index (MRI)</th>
<th>Carotid IMT (ultrasound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV LCAT mutation carriers ($n=36$)§</td>
<td>0.54 ($p&lt;0.001$)</td>
<td>0.50 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>PWV matched controls ($n=36$)§</td>
<td>0.58 ($p&lt;0.001$)</td>
<td>0.36 ($p=0.04$)</td>
</tr>
</tbody>
</table>

Values are Pearson’s correlation coefficients $R$, with respective $p$-values in brackets.

§ For 9 of 45 matched controls in which PWV was assessed, no MRI or ultrasound data were available. Respective matched carriers were excluded from correlation analysis, rendering 36 matched pairs.

**Figure 2** Scatter plot of PWV to IMT (carotid ultrasound) and NWI (carotid MRI)

**Figure 2a** Carotid-femoral PWV plotted to carotid IMT

Continuous line indicates correlation between PWV and cIMT in controls (open symbols; $n=36$), Pearson’s $R=0.36$ ($p=0.04$); Dashed line indicates correlation between PWV and cIMT in LCAT mutation carriers (closed symbols; $n=36$), Pearson’s $R=0.50$ ($p<0.001$).
accepted 2011). Table 2 and Figure 2 show that PWV correlated well with both cIMT and carotid NWI obtained by MRI.

**DISCUSSION**

In this study we show that aortic pulse wave velocity is increased in LCAT mutation carriers compared to controls, indicative of increased arterial stiffness in these patients. This difference retained significance in multivariate analysis and after exclusion of patients with CVD. In addition to our primary study objective, this study also reveals a strong correlation between arterial stiffness and thickness of the carotid arterial wall as assessed by ultrasound (cIMT) and 3.0 T MRI.

**Arterial stiffness in LCAT deficiency**

The observed increase in arterial stiffness in LCAT mutation carriers might result from accelerated atherosclerosis in these patients, which in turn might be caused by decreased reverse cholesterol transport from the vascular wall due to the impaired maturation of HDL. We did, however, not observe a correlation of PWV with HDL-c (not in carriers, nor in controls), although this might be explained by large standard deviations in both parameters and small group size. Supporting our observations of increased PWV in persons exposed to lifelong low HDL-c levels, PWV was previously found to be inversely related to HDL-c levels.\(^\text{13-15}\) In a population-based
study, 122 middle-aged subjects with low HDL-c levels had significantly higher PWV independent of age, sex, physical activity and smoking status, compared to 795 subjects with normal HDL-c levels. In a cross-sectional study among postmenopausal women, HDL-c was also reported to be inversely and independently related to PWV. However, neither of these two studies adjusted for blood pressure, an important determinant of PWV. A recent population based study in China showed that, after correction for traditional cardiovascular risk factors including blood pressure, PWV was inversely correlated with HDL-c. In our study, LCAT mutation carriers with low HDL-c levels exhibited increased PWV even after correction for blood pressure.

We identified a slight increase in BMI in carriers. However, in multivariate analysis, BMI did not affect the relation between LCAT genotype and PWV. This is in accordance with a systematic review, indicating that risk factors other than age and blood pressure contribute only modestly to arterial stiffness.

We can only speculate whether increasing HDL-c in LCAT mutation carriers would lead to reversal of increased arterial stiffness. In carriers of ABCA1 mutations, who also suffer from low HDL-c levels, endothelial function of the brachial artery as assessed by flow mediated dilation was reduced and infusion of reconstituted HDL-c (rHDL-c) restored endothelial function in these patients. Since endothelial function and arterial stiffness seem correlated, infusion of rHDL-c might improve arterial stiffness in LCAT mutation carriers.

**Correlation of arterial stiffness to carotid wall thickening**

We observed strong relationships between arterial stiffness and carotid wall thickness, as a measure of atherosclerosis. This is in line with previous observations in the general population, hypertensive patients and in patients with type 2 diabetes mellitus. There is discussion about the nature of the relationship between arterial stiffness and atherosclerosis. Arterial stiffness might simply reflect the burden of atherosclerotic plaque in the arterial wall. However, the relationship might be causal: arterial stiffness might promote atherosclerosis by causing: 1) increased wave reflection, leading to elevated central aortic systolic pressure and pulse pressure which in turn increases left ventricular work load and (2) altered hemodynamics and shear stress, stimulating the formation of atherosclerotic plaques. This first report of a strong relation between PWV and arterial wall thickness assessed by MRI underscores the interrelation between arterial stiffness and arterial wall thickening and fuels the discussion about the role of arterial stiffness in atherogenesis. Large prospective studies should further address the exact relationship between arterial stiffness and atherosclerosis.

**Strengths and weaknesses** Several aspects of our study merit closer consideration. An important strength of our study is that LCAT mutation carriers were individually matched with controls and that LCAT mutation carriers who were referred to our outpatient clinic with a history of CVD to minimize potential referral bias. We only included carriers identified in families...
of which the probands were asymptomatic for cardiovascular disease. Furthermore, the use of statins and antihypertensive medication is considerably higher in LCAT mutation carriers, and since both statins\textsuperscript{22-25} and antihypertensive drugs\textsuperscript{26} have shown to decrease PWV, the actual difference in PWV might even be larger between carriers and controls.

Our study has the following limitations. First, compared to controls, carriers show increased levels of plasma triglycerides, a finding that has also been reported by others\textsuperscript{27} and which is possibly caused by increased de novo lipogenesis in the liver\textsuperscript{28}. However, in a recent population-based study, PWV was not associated with triglycerides\textsuperscript{15}, making it unlikely that the increase in triglycerides contributes to the increased PWV in carriers. Secondly, since carriers had a higher BMI it is possible they might have lower levels of physical activity and higher fat intake. We did not assess this and therefore have not corrected for physical activity or diet.

**Perspectives** Since carriers of LCAT mutations characterized by low HDL-c levels have increased arterial stiffness compared to unaffected controls, we consider that this study supports close clinical monitoring of cardiovascular risk factors in carriers of LCAT mutations. Because PWV is a reproducible, non-invasive and readily applicable functional measure of arterial stiffness, it may be an useful method to assess and monitor the increased CVD risk in these patients.

In addition to statins and antihypertensive medication, increasing HDL-c through lifestyle modifications or pharmacological treatment might also reduce arterial stiffness by improvement of cholesterol efflux, reduction of inflammation or improvement of endothelial function\textsuperscript{13, 29, 30}. In future studies, it would be of interest to examine whether treatment specifically aimed at increasing HDL-c will affect arterial stiffness. Also, our data bolsters the notion that LCAT might be an interesting target to reduce cardiovascular risk and supports LCAT enhancing strategies currently evaluated in preclinical studies\textsuperscript{31-33}. 
REFERENCES


