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Beta2-adrenergic activity modulates vascular tone regulation in lecithin:cholesterol acyltransferase knockout mice

S. Manzini [a], C. Pinna [a], M. Busnelli [a], P. Cinquanta [a], E. Rigamonti [a], G.S. Ganzetti [a], F. Dellera [a], A. Sala [a,b], L. Calabresi [a], G. Franceschini [a], C. Parolini [a,1], G. Chiesa [a,*,1]

[a] Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy
[b] Institute of Biomedicine and Molecular Immunology, CNR, Palermo, Italy

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ABSTRACT

Lecithin:cholesterol acyltransferase (LCAT) deficiency is associated with hypoalphalipoproteinemia, generally a predisposing factor for premature coronary heart disease. The evidence of accelerated atherosclerosis in LCAT-deficient subjects is however controversial. In this study, the effect of LCAT deficiency on vascular tone and endothelial function was investigated in LCAT knockout mice, which reproduce the human lipoprotein phenotype. Aortas from wild-type ($Lcat^{wt}$) and LCAT knockout ($Lcat^{KO}$) mice exposed to noradrenaline showed reduced contractility in $Lcat^{KO}$ mice ($P < 0.005$), whereas acetylcholine exposure showed a lower NO-dependent relaxation in $Lcat^{KO}$ mice ($P < 0.05$). Quantitative PCR and Western blotting analyses suggested an adequate eNOS expression in $Lcat^{KO}$ mouse aortas. Real-time PCR analysis indicated increased expression of β2-adrenergic receptors vs wild-type mice. Aorta stimulation with noradrenaline in the presence of propranolol, to abolish the β-mediated relaxation, showed the same contractile response in the two mouse lines. Furthermore, propranolol pretreatment of mouse aortas exposed to L-NAME prevented the difference in responses between $Lcat^{wt}$ and $Lcat^{KO}$ mice. The results indicate that LCAT deficiency leads to increased β2-adrenergic relaxation and to a consequently decreased NO-mediated vasodilation that can be reversed to guarantee a correct vascular tone. The present study suggests that LCAT deficiency is not associated with an impaired vascular reactivity.

1. Introduction

Lecithin:cholesterol acyltransferase (LCAT) is a 63 kDa glycoprotein which catalyzes the transfer of a fatty acid from the *sn*-2 position of phosphatidylcholine to the 3-hydroxyl group of cholesterol, generating cholesteryl esters and lysolecithin [1]. In blood, LCAT preferentially binds to high density lipoproteins (HDL) and, by esterifying free cholesterol, it converts discoidal preβ-HDL into mature, spherical α-migrating HDL, thus playing a key role in the metabolism of this lipoprotein class [2].

Loss-of-function mutations at both alleles of the human LCAT gene result either in familial LCAT deficiency or in fish eye disease, two very rare metabolic disorders characterized by severe hypoalphalipoproteinemia [3]. HDL deficiency is generally expected to be associated with increased coronary heart disease risk [4,5]. However, premature coronary heart disease is not a consistent finding among individuals with LCAT deficiency [6–8]. Moreover, the measurement of carotid intima media thickness in LCAT-deficient subjects, compared to matched controls, suggests that LCAT deficiency has a modest if no effect on atherosclerosis development [9–11].

The impact of LCAT in atherosclerosis has also been investigated in genetically modified models with inconclusive results. Targeted deletion of LCAT in mice reproduces the lipoprotein phenotype found in LCAT-deficient subjects, with extremely low HDL-cholesterol levels and increased pre-β HDL vs control mice [12,13]. When, however, for atherosclerosis studies, LCAT knockout mice were cross-bred into atherosclerosis-prone mouse lines, such as apoE knockout or LDLR-deficient mice, contradictory results were obtained [14,15]. A possible explanation resides in the fact that, depending on the genetic background or the dietary treatment adopted, LCAT deletion differently affected apoB-containing lipoprotein levels, leading to reduced or increased atherosclerosis development.

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoproteins; NA, noradrenaline; ACh, acetylcholine; L-NAME, NG-nitro-L-arginine methyl ester; SNP, Sodium nitroprusside; NO, nitric oxide; eNOS, endothelial NO synthase; $Lcat^{KO}$, LCAT knockout mice.

* Corresponding author at: Department of Pharmacological and Biomolecular Sciences, via Balzaretti 9, 20133 Milano, Italy.
E-mail addresses: stefano.manzini@gmail.com (S. Manzini), christian.pinna@unimi.it (C. Pinna), marco.busnelli@gmail.com (M. Busnelli), paola.cinquanta84@gmail.com (P. Cinquanta), rigamonti.elena@hsr.it (E. Rigamonti), giuliaganzetti@gmail.com (G.S. Ganzetti), dellera.federica@gmail.com (F. Dellera), angelo.sala@unimi.it (A. Sala), laura.calabresi@unimi.it (L. Calabresi), guido.franceschini@unimi.it (G. Franceschini), cinzia.parolini@unimi.it (C. Parolini), Giulia.Chiesa@unimi.it (G. Chiesa).
[1] These authors contributed equally to this study.

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Several *in vitro* and *in vivo* studies have highlighted the ability of HDL to preserve the vascular tone by inducing the release of endothelial vasoactive molecules, thus protecting from atherosclerosis development [16–18]; however, the impact of hypoalphalipoproteinemia on endothelial function has been poorly addressed [19] and a possible effect of the hypoalphalipoproteinemia driven by LCAT deficiency on the vascular tone has not been explored yet. In the present study, the effect of LCAT deletion on vascular reactivity was investigated in LCAT knockout mice fed chow diet. Interestingly, differences in vascular reactivity to both contractile and relaxant stimuli were detected in LCAT knockout mouse aortas.

2. Materials and methods

2.1. Materials

Norepinephrine hydrochloride, Angiotensin II, NG-nitro-L-arginine methyl ester, acetylcholine, sodium nitroprusside, and D,L-propranolol were purchased from Sigma-Aldrich. All compounds were freshly dissolved in distilled H_2O.

2.2. Animals

Procedures involving animals and their care were conducted in accordance with institutional guidelines that are in compliance with national (D.L. No. 26, March 4, 2014, G.U. No. 61 March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011).

C57BL/6J male mice, aged 4–6 months, wild type ($Lcat^{wt}$) or homozygous for a targeted disruption of LCAT gene ($Lcat^{KO}$) (kindly provided by Prof. J.S. Parks) [13], and mice deficient of murine apoA-I ($A\text{-}I^{KO}$) [20] have been enrolled for the study. Mice were housed at constant temperature and relative humidity, and fed a commercial standard diet (Mucedola, Italy).

2.3. Vascular reactivity study

Mice were sacrificed by cervical dislocation. Thoracic aortas were carefully excised, cleaned of fat and connective tissue, and cut into 2–3 mm rings. Ring segments were suspended in 5-ml organ baths containing Krebs' solution at 37 °C continuously bubbled with 95% O_2:5% CO_2. The Krebs' solution had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.1, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 5.5; pH 7.4. Ring segments were mounted horizontally in the organ bath by inserting 2 tungsten wires (50 μm) through the lumen of the vessel, one wire attached to a rigid support and the other to an isometric force transducer (Fort 10, World Precision Instruments, Sarasota, FL) for tension recording. Mechanical activity was displayed and recorded with a digital recording system (PowerLab 8SP — AD Instruments, Basile, Comerio, Italy). Tissues were equilibrated for 1 h, and loaded to a tension of 0.8 g. A slight reduction in this value occurred after the equilibration period.

At the end of the equilibration period, each preparation was exposed to noradrenaline EC_{60} (NA 10^{-7} M) until two reproducible contractions were obtained. Cumulative concentration–response curves to NA (10^{-9}–10^{-5} M) and to Angiotensin II (Ang-II, 10^{-9}–10^{-5} M) were obtained at resting tone. Contractile response to NA was expressed as mN/mg tissue. Cumulative concentration–response curves to: acetylcholine (ACh, 10^{-9}–3×10^{-6} M), NG-nitro-L-arginine methyl ester (L-NAME, 10^{-6}–10^{-4} M) and Sodium nitroprusside (SNP, 3×10^{-11}–3×10^{-7} M) were run in preparations precontracted with NA (10^{-7} M).

After each concentration–response curve, tissues were washed several times with fresh Krebs' solution and left in the bath (for about 1 h) until the resting tone had recovered. Contractile response to NA and cumulative concentration–response curves to L-NAME were also assessed in aortic rings pretreated with the selective β-adrenergic receptor antagonist D,L-propranolol (10^{-6} M).

2.4. Aorta histology

After the *ex-vivo* evaluation of vascular reactivity, the same aortic rings were embedded in OCT compound (Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands). Serial cryosections (7 μm thick) of the aorta were cut and stained with hematoxylin and eosin (Bio-Optica, Milano, Italy). The Aperio ScanScope GL Slide Scanner (Aperio Technologies, Vista, CA, USA) equipped with a Nikon 20×/0.75 Plan Apochromat objective producing a 0.25 μm/pixel scanning resolution with a 40× magnification was used to acquire images. The Aperio ImageScope software (version 8.2.5.1263) was used to evaluate tunica media area and thickness, internal and external elastic lamina perimeter as well as the number of elastic laminae. An operator blinded to genotype acquired and evaluated the images.

2.5. qPCR analyses

Aortas (thoracic to iliac arteries bifurcation) were collected from anesthetized animals (isoflurane, Baxter, Italy) after perfusion with PBS, cleaned of excess fat and snap-frozen in liquid nitrogen. Total RNA was isolated using the NucleoSpin RNA extraction kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. RNA concentration and purity were estimated evaluating the ratio of optical density at 260 and 280 nm (Nanodrop 1000, ThermoScientific, Wilmington, DE). RNA integrity was checked by electrophoresis in a 1.7% TAE gel stained with ethidium bromide (Sigma-Aldrich, Seelze, Germany). Total RNA (1 μg) was reverse transcribed with random hexamer primers and MultiScribe reverse transcriptase (Life Technologies, Carlsbad, CA) following the manufacturer's instructions. cDNA (20 ng) was quantified by qPCR on a CFX Connect 96 thermal cycler by using an iTaq Universal SYBR® Green Supermix (Biorad, Segrate, Italy) and specific primers indicated in Table 1. Efficiency and melting curve were calculated for each primer pair. Expression data are relative

Table 1
Sequences of the primers used for real-time PCR analysis.

Gene	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)
α1a	TGCGAGGACTGAAGGTCCGC	CAGGGACGCTGGGCGAATGG
α2a	TGCTGGTTGTTGTGGTTGTT	GGGGGTGTGGAGGAGATAAT
β1	GAAGGCGCTCAAGACACTGG	CCAGGTCGCGGTGGAA
β2	TCTGTCTGTCTGTCTGGATGATG	CCAGGTCGCGGTGGAA
β3	GGCAACCTGCTGGTAATCAT	TCCACTGACGTCCACAGTTC
AKT	GATCAAGATGACAGCATGGAGTGT	GGCAATGCAGAGGAGCGT
CAT	CCTCGTTCAGGATGTGGTTT	TCTGGTGATATCGTGGGTGA
Cyclophilin	AGCACTGGGGAGAAAGGATT	AGCCACTCAGTCTTGGCAGT
Edn1	CCTGGACATCATCTGGGTC	TGTGGCCTTATTGGGAAG
eNOS	ACAAATAGAGGCAATCTTCGTTCA	CTATAGCCCGCATAGCGTATCA
GTPCH1	GCAGCGAGGAGGAAAACCA	CCAGCGAGAGCAGAATGGA
HMOX1	GCTAGCCTGGTGCAAGATACT	GCCAACAGGAAGCTGAGAGTG
iNOS	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG
M3	CACGGCTGCCAGATATGACC	TGGTCACTTGGTCAGAACGC
NOX1	CTGACAAGTACTATTACACGAGAG	CATATATGCCACCAGCTTATGG AAG
NOX2	AACTGTATGCTGATCCTGCTGC	GTTCTCATTGTCACCGATGTCAG
NOX4	TGAGGAGTCACTGAACTATGAAGT TAATC	TGACTGAGGTACAGCTGGATGT TCACA
p47PHOX	ACCGGCTATTTCCCATCC	TGGATCCTCTGTGCGTTG
p67PHOX	CTCTACTACAGAATGGAGAAGT ACG	GCCCCAGGATCTTGTAGTCTAT
Scarb1	AGCGTGGACCCTATGTCTACA	CCATGCGACTTGTCAGGCT
SOD1	ACCAGTGCAGGACCTCATTTTAA	TCTCCAACATGCCTCTCTTCATC
SOD2	CACATTAACGCGCAGATCATG	CCAGAGCCTCGTGGTACTTCTC
SOD3	GCTTCGACCTAGCAGACAGG	GTCGTCCTAGCTCCATCCAG
XDH	AAAGGACCAGACGATTGCTCC	TCACACGTTCCCCTTCAAAAC