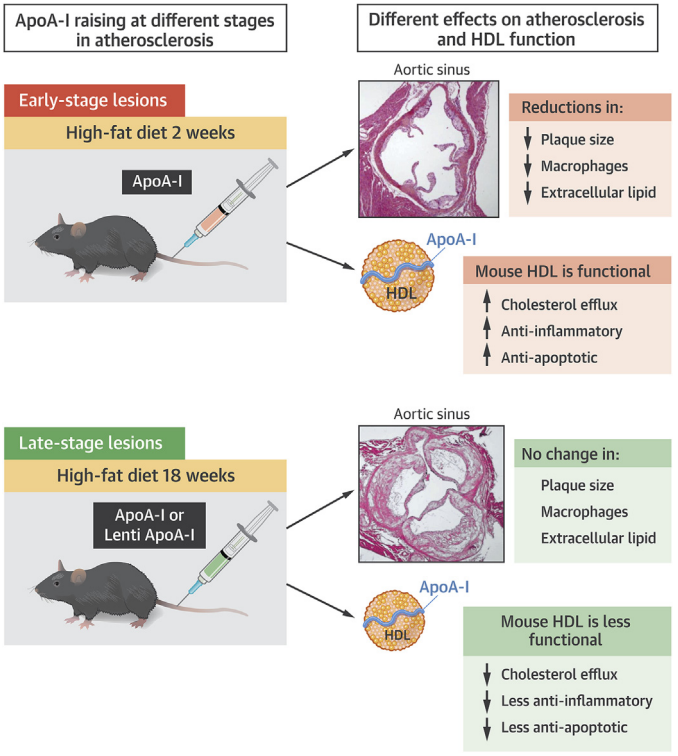
JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 3, NO. 2, 2018


PRECLINICAL RESEARCH

# Strikingly Different Atheroprotective Effects of Apolipoprotein A-I in Early- Versus Late-Stage Atherosclerosis

Jamie Morton, MBBS, PhD,[a,b] Shisan Bao, MD, PhD,[c] Laura Z. Vanags, BSc, PhD,[a,b] Tania Tsatralis, BSc,[a] Anisyah Ridiandries, BSc, PhD,[a,b] Chung-Wah Siu, MBBS, MD,[d] Kwong-Man Ng, BSc, PhD,[d] Joanne T.M. Tan, BSc, PhD,[a,b] David S. Celermajer, MBBS, PhD,[a,b,e] Martin K.C. Ng, MBBS, PhD,[a,b,e] Christina A. Bursill, BSc, PhD[a,b]

## VISUAL ABSTRACT




Morton, J. et al. J Am Coll Cardiol Basic Trans Science. 2018;3(2):187–99.

## HIGHLIGHTS

- **The atheroprotective effects of apoA-I are dependent on the plaque stage from which apoA-I is infused.**
- **The atheroprotective effects of apoA-I infusions are also impaired in older mice with a greater disease milieu.**
- **Ex vivo studies with mouse HDL found an impairment in HDL functionality with increasing disease/age of the mice as well as a reduced ability of apoA-I infusions to improve the atheroprotective functions of HDL.**
- **Our study provides understanding regarding the disparity between the very positive results of HDL/apoA-I raising in preclinical studies, largely performed in younger animals with early-stage disease, and the large-scale HDL-raising clinical trials in more elderly patients with established plaque that have failed to show benefit.**

From the [a]Immunobiology Group, The Heart Research Institute, Sydney, Australia; [b]Department of Medicine, Sydney Medical School, University of Sydney, Sydney, Australia; [c]Discipline of Pathology, University of Sydney, Sydney, Australia; [d]Division of Cardiology, Department of Medicine, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong; and the [e]Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia. This work was supported by a post-graduate scholarship from the National Medical Research Council of Australia (NHMRC) (#598402) and the Heart Foundation (HF) [PC09S4757] to Dr. Morton; and the NHMRC Project Grant (#632512) to Drs. Ng and Bursill. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Ng and Bursill contributed equally to this work.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2017.11.004

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APRIL 2018:187–99

**ABBREVIATIONS AND ACRONYMS**

**apoA-I** = apolipoprotein A-I

***apoE*$^{-/-}$** = apolipoprotein E deficient

**Bcl-xL** = B-cell lymphoma-extra large

**HCAEC** = human coronary artery endothelial cell

**HDL** = high-density lipoprotein

**HFD** = high-fat diet

**LDL** = low-density lipoprotein

**LVApoAI** = lentivirus overexpressing apolipoprotein A-I

**LVGFP** = lentivirus overexpressing green fluorescence protein

**MCP** = monocyte chemoattractant protein

**micro-CT** = micro-computed tomography

**rHDL** = reconstituted high-density lipoprotein

**SAA** = serum amyloid amylase

**SMC** = smooth muscle cell

**SNP** = single-nucleotide polymorphism

**TNF** = tumor necrosis factor

**VCAM** = vascular cell adhesion molecule

**SUMMARY**

Preclinical studies have shown benefit of apolipoprotein A-I (apoA-I)/high-density lipoprotein (HDL) raising in atherosclerosis; however, this has not yet translated into a successful clinical therapy. Our studies demonstrate that apoA-I raising is more effective at reducing early-stage atherosclerosis than late-stage disease, indicating that the timing of HDL raising is a critical factor in its atheroprotective effects. To date, HDL-raising clinical trials have only been performed in aged patients with advanced atherosclerotic disease. Our findings therefore provide insight, related to important temporal aspects of HDL raising, as to why the clinical trials have thus far been largely neutral. (J Am Coll Cardiol Basic Trans Science 2018;3:187-99) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Epidemiological research has established a strong inverse association between high-density lipoprotein (HDL) level and atherosclerosis (1). Similar inverse associations have also been shown for apolipoprotein A-I (apoA-I), the main protein constituent of HDL. Numerous preclinical studies have supported the epidemiological data with animal models of atherosclerosis demonstrating HDL/apoA-I interventions reduce plaque size and inflammation (2-6). Studies using infusions of reconstituted HDL (rHDL) have been promising in short-term clinical intervention studies (7,8); however, contemporary HDL-raising clinical trials using cholesterol ester transfer protein (CETP) inhibition or niacin have not yet shown benefit on clinical endpoints (9-11) or imaging assessment of atherosclerotic plaque (12-14). Recently, a third CETP inhibitor (evacetrapib) was discontinued due to lack of efficacy. Off-target pharmacological effects such as increased systolic blood pressure have been reported, yet it invites reflection on how to reconcile the neutral results of the clinical trials with the mountain of evidence supporting the atheroprotective benefits of HDL.

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There are notable differences in the timing of the HDL/apoA-I interventions between the preclinical studies and the recent clinical trials (7,12-14). Patients in clinical trials have had established atherosclerotic disease, with the average patient age past midlife. By contrast, the majority of preclinical HDL/apoA-I raising studies are in young animals with early to midstage plaques (2-4). Mechanistically, the atheroprotective effects of HDL primarily involve the suppression of key events in the early stage of plaque development. For example, HDL and apoA-I inhibit early-stage inflammatory markers such as adhesion molecule and cytokine/chemokine expression (15,16), low-density lipoprotein (LDL) oxidation (17), and monocyte chemotaxis (16). HDL also confers atheroprotective effects in midstage plaques by promoting the efflux of cholesterol from foam cell macrophages (18). By contrast, there are fewer known atheroprotective mechanisms for HDL in late-stage atherosclerosis, in which the plaques have strikingly different features and compositions such as a necrotic core (19) that may not be amenable to cholesterol efflux. There is also evidence that HDL functionality is compromised in aged patients (20,21) with coronary artery disease (22,23).

The timing of HDL/apoA-I raising may therefore be critical for HDL to exert its maximum beneficial effects. We report that apoA-I raising, when initiated in young animals with early-stage disease, decreased atheroma progression and improved plaque composition. By contrast, these beneficial effects were greatly attenuated in older mice with late-stage disease, and HDL functionality was compromised. Our findings may explain the disparity between the preclinical studies and recent human trials using HDL-raising therapies.

## METHODS

An expanded Methods section is available in the Supplemental Appendix.

All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

Manuscript received August 22, 2017; revised manuscript received November 3, 2017, accepted November 4, 2017.