A Novel Approach to Management of Cardiovascular Disease in Patients with Familial Hypercholesterolemia

Dr Sam Mirzaee

MD, MPH, FRACP

A thesis submitted for the degree of
Doctor of Philosophy at Monash University in 2019

Monash Cardiovascular Research Centre,

Department of Medicine, School of Clinical Sciences at

Monash Health, Monash University

Melbourne, Australia

Copyright notice

ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death in Australia and worldwide. Familial hypercholesterolemia (FH) is a common autosomal dominant genetic disorder of lipoprotein metabolism resulting in elevated serum low-density lipoprotein cholesterol (LDL-C) levels leading to substantial increased risk for early onset cardiovascular diseases. Current evidence shows that despite guidelines and protocolizing FH screening, the majority of individuals with FH remain undiagnosed and undertreated.

The aims of the thesis are first to outline the current evidence of the impact of intensive lipid lowering therapy on atherosclerotic coronary disease in high risk patients and FH (Chapter-2), to assess the prevalence and prognosis of patients with FH and acute coronary syndrome in a cardiac care unit (Chapter 3-A), to evaluated the knowledge gap and awareness of FH among health professionals involved in the management of coronary disease (Chapter3-B), to determine a novel avenue in screening patients with FH in the hospital (Chapter 3-C), to review vascular inflammation using pericoronary adipose tissue attenuation (PCAT) in CT coronary angiogram (CTCA) as a non-invasive risk stratifying tool in patients with FH (Chapter-4), and finally to evaluate post prandial hyperlipaemia by using a simplified oral fat tolerance test to optimise risk stratification in patients with FH (Chapter-5).

Eight manuscripts from this body of research (five original scientific studies, two systematic reviews & meta-analysis articles, one review article) have been published or submitted for publication in international peer reviewed journals.

The salient findings of this thesis are: **a)** FH is relatively common among patients with premature coronary artery disease (CAD), **b)** there are substantial gaps in the knowledge and awareness of FH among healthcare providers involved in the management of acute coronary syndrome, **c)** a high yield from opportunistic screening for FH in a tertiary hospital laboratory with a prevalence at least analogous to the general population, **d)** greater degree of

vascular inflammation assessed by PCAT in CTCA in FH, **e)** significantly higher post prandial hyperlipaemia in FH subjects with CAD, and finally **f)** invasive lipid lowering therapy will improve the course of coronary disease and further cardiovascular outcomes in high risk patients and FH.

We concluded there remains an unmet need for optimising detection and management of patients with FH by a multifaceted approach. A focused education and clinical training in healthcare providers working in cardiac care units is warranted to raise their awareness of FH. Our findings support the benefit of establishing an efficient “alert system” in hospital laboratories with a trigger “reflex testing” to facilitate screening for index FH. Since, there is heterogeneity among FH patients there is a need to stratify the risk of CAD to tailor an efficient management plan. Non-invasive assessment of vascular inflammation by PCAT, and detecting post prandial hyperlipaemia by OFTT may enhance risk stratification and guide combination therapy in FH patients to improve their clinical outcome.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature:

Print Name: Dr Sam Mirzaee

Date: 23rd November 2019

Publications During Enrolment

1. *Nelson AJ, **Mirzaee S,** Nicholls SJ. High-Dose Omega-3 Fatty Acids in Cardiovascular Prevention: Finally Living Up to Their Potential? Am J Cardiovasc Drugs. 2019 Sep 4, 1179-187.*

2. ***Mirzaee S**, Cameron JD, Nicholls SJ. The Role of Nutrition and Environmental Factors in Management of Familial Hypercholesterolemia; ACNEM Journal Vol 38 No 2 –2019 July,5-7.*

3. ***Mirzaee S**, Choy KW, Doery JCG, Zaman S, Cameron JD, Nasis A. The tertiary hospital laboratory; a novel avenue of opportunistic screening of familial hypercholesterolemia. Int J Cardiol Heart Vasc. 2019 May, 3;23:100354.*

4. *Macpherson H, Brownell S, Duckham RL, Meyer B, **Mirzaee S**, Daly RM. Multifaceted intervention to enhance cognition in older people at risk of cognitive decline: study protocol for the Protein Omega-3 and Vitamin D Exercise Research (PONDER) study. BMJ Open. 2019 May 9;9(5): ;e024145.*

5. *Lin A, Nerlekar N, Rajagopalan A, Yuvaraj J, Modi R, **Mirzaee S**, Munnur RK, Seckington M, Doery JC, Seneviratne S, Nicholls SJ, Wong DT. Remnant cholesterol and coronary atherosclerotic plaque burden assessed by computed tomography coronary angiography. Atherosclerosis. 2019 May, 284: 24-30.*

6. *Mehta OH, Cameron JD, **Mirzaee S**. Familial Hypercholesterolemia with Coexisting Renovascular Stenosis and Premature Coronary Artery Disease. Am J Hypertens. 2019 Jul 17; 32(8): 730-733.*

7. *Thein PM, Ong J, Crozier TM, Nasis A, **Mirzaee S**, Tan SX, Junckerstorff R.*

Predictors of acute hospital mortality and length of stay in patients with new-onset atrial fibrillation: a first-hand experience from a medical emergency team response provider. Intern Med J. 2019 Aug 49(8): 969-977.

8. Nogic J, Thein P, Mirzaee S, Comella A, Soon K, Cameron JD, West NEJ, Brown AJ. Biodegradable-polymer versus polymer-free drug eluting stents for the treatment of coronary artery disease. Cardiovasc Revasc Med. 2019 OCT ;20(10): 865-870

9. ***Mirzaee S**, Cameron JD. Coronary computed tomography angiogram in familial hypercholesterolemia: A double edge sword. J Clin Lipidol. 2018 Nov - Dec; 12(6): 1549.*

10. ***Mirzaee S**, Thein PM, Nogic J, Nerlekar N, Nasis A, Brown AJ. The effect of combined ezetimibe and statin therapy versus statin therapy alone on coronary plaque volume assessed by intravascular ultrasound: A systematic review and meta-analysis. J Clin Lipidol. 2018 Sep – Oct; 12(5): 1133-1140.*

11. ***Mirzaee S**, Cameron JD. Familial hypercholesterolemia and cardiovascular risk stratification. J Clin Lipidol. 2018 Sep – Oct; 12(5) :1328-1329.*

12. ***Mirzaee S**, Thein PM, Wong D, Nasis A. A Small Change Can Make a Big Difference: A Lesson from Evolocumab. Heart Lung Circ. 2019 Mar 28(3):e21-e22.*

13. *Thein PM, White K, Banker K, Lunny C, **Mirzaee S**, Nasis A. Preoperative Use of Oral Beta-Adrenergic Blocking Agents and the Incidence of New-Onset Atrial Fibrillation After Cardiac Surgery. A Systematic Review and Meta-Analysis. Heart Lung Circ. 2018 March;27(3):310-321.*

14. *Nogic J, Thein PM, Cameron J, **Mirzaee S**, Ihdayhid A, Nasis A. The utility of personal activity trackers (Fitbit Charge 2) on exercise capacity in patients post acute coronary syndrome [UP-STEP ACS Trial]: a randomised controlled trial protocol. BMC Cardiovasc Disord. 2017 Dec 29;17(1):303.*

Unpublished Work

1. **Familial Hypercholesterolemia and Incidence of Fatal Ventricular Arrhythmias Complicating Premature Acute Coronary Syndrome.**
   Sam Mirzaee MD, MPH[1], Andrew Lin MBBS, BMed Sci[1], James D Cameron MBBS MD BE MEngSc[1] (Elec).[1] *Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia.*

2. **Assessment of Vascular Inflammation by Pericoronary Adipose Tissue Attenuation using Computed Tomography in patients with familial hypercholesterolemia.** Sam Mirzaee [1], Udit Thakur[1], Andrew Lin[1,] Nitesh Nerlekar[1], James D Cameron[1,] Arthur Nasis, Stephen J Nicholls[1], Dennis TL Wong[11] *Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Monash University, Melbourne, Australia.*

3. **Tailoring a Novel Cardiometabolic Risk Stratification in Patients with Familial Hypercholesterolemia by Adding Oral Fat Tolerance Test.** *Sam Mirzaee[1], James CG Doery[2], Arthur Nasis[1], and James D Cameron[.1] [1] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia,[2] Monash Health Pathology, Monash Health, Monash University, Melbourne, Australia.*

4. **The role of low density lipoprotein apheresis in the management of heterozygous familial hypercholesterolemia: A systematic review and meta-analysis.** *Sam Mirzaee MD MPH[a], Paul M Thein MBBS[a] , Sarah Zaman MBBS, PhD[a], James D Cameron MBBS MD BE (Elec) MEngSc[a]. Arthur Nasis MBBS, MD, PhD[a]*
   [a] Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Monash University, Melbourne, Australia. *(Under minor revision by Journal of Clinical Apheresis)*

5. **New Era in the Management of Atherosclerosis; Current and Emerging Novel Lipid Modulators.** Sam Mirzaee[1], James CG Doery[2], Arthur Nasis[1], Dennis TL Wong[1], James D Cameron[1]. *[1] Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Monash University, Melbourne, Australia. [2] Monash Health Pathology, Monash Health, Melbourne, Australia.*

6. **Impact of Side Branch Pre-Dilation on Angiographic Outcomes in Provisional Stent Strategy in Non-Left Main Coronary Bifurcation Lesions: a Systematic Review and Meta-Analysis** *Sam Mirzaee MD, MPH; Mourushi Isa; Udit Thakur MBBS; James D Cameron1 MBBS MD BE (Elec) MEngSc; Benjamin K Dundon MBBS, PhD. (Accepted by Journal of Invasive Cardiology)*

7. **Arrhythmia Paradox in Patients with Familial Hypercholesterolemia**

   Sam Mirzaee MD, MPH[1], James D Cameron MBBS MD BE (Elec) MEngSc[1]

   [1] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia. *(Accepted by Journal of clinical lipidology)*

Presentations at National and International Scientific Meetings

Cardiac society of Australia and New Zealand, Perth, Australia, August 2017

European Society Cardiology Congress, Barcelona, Spain, September 2017

3rd World Congress in Clinical Lipidology, Brisbane, Australia, February, 2017

Australian Atherosclerosis Society, Sydney, Australia, October, 2017

Cardiac society of Australia and New Zealand, Brisbane, Australia, August 2018

European Society Cardiology Congress, Munich, Germany, September 2018

Transcatheter Cardiovascular Therapeutics (TCT), San Diego, September 2018

Cardiac Society of Australia and New Zealand, Adelaide, Australia, August 2019

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes *three* original papers published in peer reviewed journals, two original papers under minor revision and three submitted publications. The core theme of the thesis is a novel approach to management of cardiovascular disease in patients with familial hypercholesterolemia. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student working within the Monash Cardiovascular Research Centre under the supervision of Professor James D Cameron.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of four chapters my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status *(published, in press, accepted or returned for revision, submitted)*	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
Chapter 2A	New Era in the Management of Atherosclerosis; Current and Emerging Novel Lipid Modulators	*Submitted*	*70%. Concept and collecting data and writing first draft*	1) James CG Doery input into Data analysis manuscript 5%. 2) Arthur Nasis, input into manuscript 5%. 3) Dennis Wong input into manuscript 10%. 4) James Cameron Input into manuscript 10%.	*No* *No* *No* *No*
Chapter 2B	The role of low density lipoprotein apheresis in the management of heterozygous familial hypercholesterolemia : A systematic review and meta-analysis	Accepted	75% *Concept and collecting data and writing first draft*	1) Paul Thein, input into Data analysis manuscript 10% 2) Sarah Zaman, input into manuscript 5% 3) James D Cameron input into manuscript 5% 4) Arthur Nasis input into manuscript 5%	*No* *No* *No* *No*
Chapter 2C	*The effect of combined ezetimibe and statin therapy versus statin therapy alone on coronary plaque volume assessed by intravascular ultrasound: A systematic review and meta-analysis*	Published	*70%. Concept and collecting data and writing first draft*	*1) Paul Thein, input into manuscript 10%* *2) Jason Nojic, Data analysis, input into manuscript 5%* *3) Nitesh Nerlekar input into manuscript 5%* *4) Arthur Nasis input into manuscript 5%* *5) Adam Brown input into manuscript 5%*	No No Yes No No

Chapter 3A	Awareness of Familial Hypercholesterolemia Among Healthcare Providers Involved in the Management of Acute Coronary Syndrome in Victoria, Australia	Published	*65%. Concept and collecting data and writing first draft*	1) Hashrul N Rashid, input into manuscript 5%. 2) Odgerel Tumur input into manuscript 5%. 3) Jason Nogic input into manuscript 5%. 4) James D Cameron, input into data and manuscript 10%. 5) Stephen J. Nicholls, input into manuscript 5%. 6) Arthur Nasis input into manuscript 5%.	Yes No No No No No
Chapter 3B	Familial Hypercholesterolemia and Incidence of Fatal Ventricular Arrhythmias Complicating Premature Acute Coronary Syndrome	Under Revision	*85%. Concept and collecting data and writing first draft*	1) Andrew Lin, input into Data analysis manuscript 10%. 2) James D Cameron, input into manuscript 5%.	Yes No
Chapter 3C	The tertiary hospital laboratory; a novel avenue of opportunistic screening of familial hypercholesterolemia	Published	*75%. Concept and collecting data and writing first draft*	1) Kay W Choy input into data analysis, manuscript 5%. 2) James CG Doery input into manuscript 5%. 3) Sarah Zaman, , input into manuscript 5% 4) James Cameron Input into manuscript 5%. 5) Arthur Nasis, input into manuscript 5%.	No No No No No
Chapter 4	Assessment of vascular inflammation by Pericoronary Adipose Tissue Attenuation using Computed Tomography in patients with familial hypercholesterolemia	Submitted	*65%. Concept and collecting data and writing first draft*	1) Udit Thakur, Input into manuscript 5%. 2) Andrew Lin Input into manuscript 5%. 3) Nitesh Nerlekar input into data and manuscript 5% 4) James D Cameron; input into data and manuscript 5% 5) Arthur Nasis, input into data and manuscript 5% 6) Stephen J Nicholls; input into data and manuscript 5% 7) Dennis TL Wong;	No Yes Yes No No No No

				input into data and manuscript 5%	
Chapter 5	Tailoring a Novel Cardiometabolic Risk Stratification in Patients with Familial Hypercholesterolemia by Adding Oral Fat Tolerance Test	Submitted	*85%. Concept and collecting data and writing first draft*	1) James CG Doery input into manuscript 5%. 2) Arthur Nasis, input into data and manuscript 5% 3) James D Cameron; input into data and manuscript 5%	No No No

**If no co-authors, leave fields blank*

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 23rd November 2019

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature*:* **Date:** 23rd November 2019

ACKNOWLEDGEMENTS

I would like to thank Monash Cardiovascular Research Centre where my *“guest house”* ***** was embraced in this academic journey. I thank those filled me with intuition, those allowed me to feel both light and darkness, and provided me the opportunity to become my own catalyst. Finally I thank Professor James Cameron to whom I will be in debt for his unreserved support in this research project.

This research was supported by an Australian Government Research Training Program (RTP) Scholarship.

***** “This being human is a *guest house*.
Every morning a new arrival.
A joy, a depression, a meanness.
Some momentary awareness comes
As an unexpected visitor.
Welcome and entertain them all!
Even if they’re a crowd of sorrows,
Who violently sweep your house
Still, treat each guest honourably.
He may be clearing you out
For some new delight.
The dark thought, the shame, the malice,
Meet them at the door laughing,
And invite them in.
Be grateful for whoever comes,
Because each has been sent,
As a guide from beyond.”

~Jalal ad-Din Rumi~ (1207-1273 A.D, Persian Philosopher and Poet)

THESIS CONTENTS

Chapter 1

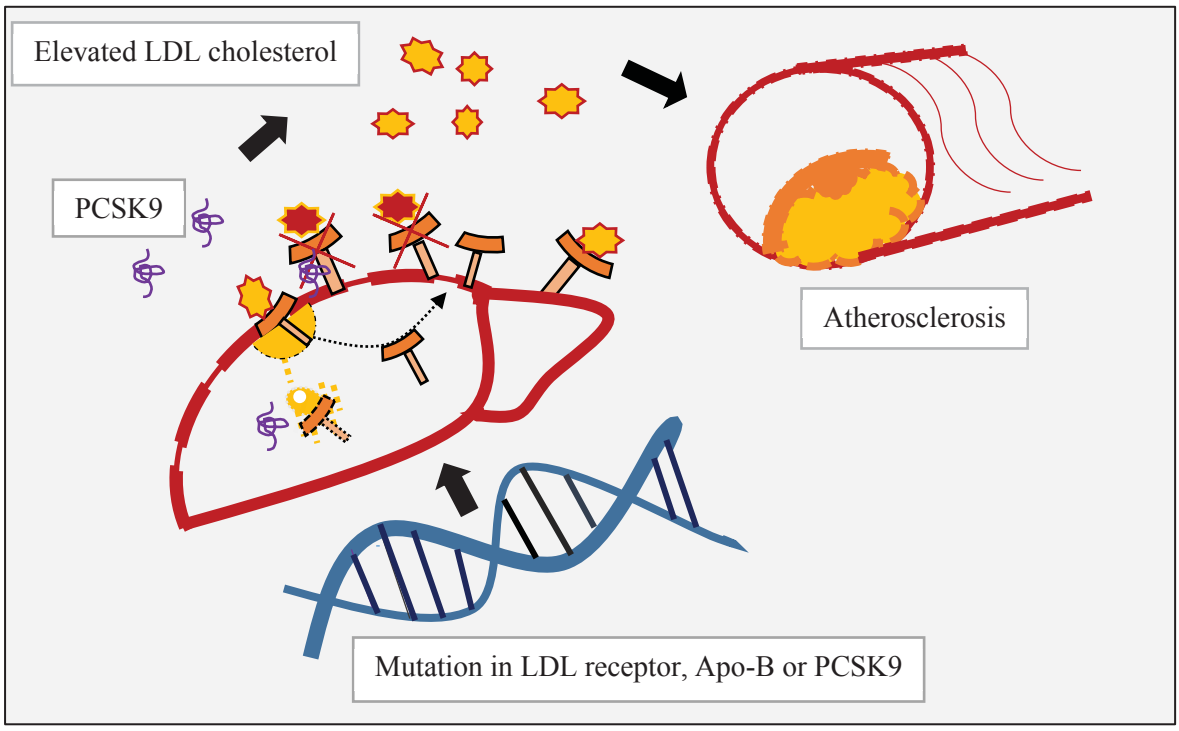
Introduction and Aims of Thesis

INTRODUCTION

A-Epidemiological characteristics and pathophysiology of familial hypercholesterolemia

Cardiovascular disease (CVD) is the leading cause of death in Australia and worldwide. Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder of lipoprotein metabolism resulting in elevated serum low-density lipoprotein cholesterol (LDL-C) levels leading to substantial increased risk for early onset cardiovascular diseases (Figure-1).[1] The prevalence of FH, formerly estimated as 1:500 in the general population, has recently been demonstrated to have a higher prevalence at approximately 1:250.[2] Current evidence shows that despite guidelines and protocolizing FH screening, the majority of individuals with FH remained undiagnosed. It has been estimated that there are between 13-34 million patients with FH globally, however only less than 1% of them are diagnosed. FH is also associated with 200,000 annual premature deaths.[3]

Figure 1-



Pathophysiological mechanism of familial hypercholesterolemia

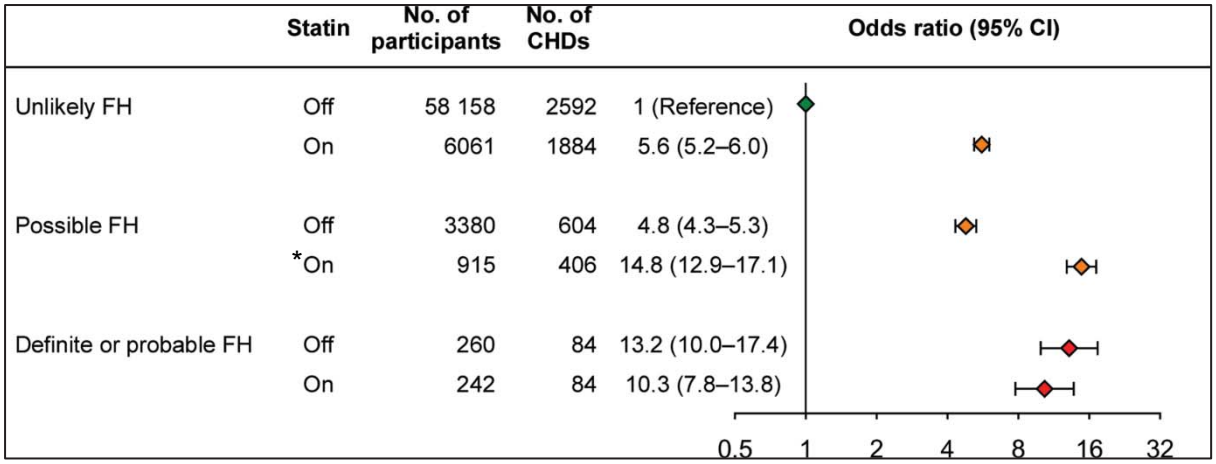


Figure 2- *The risk of coronary artery disease in patients with FH*[(1)]

*Delayed treatment with statin predominantly for secondary prevention.

The risk of cardiovascular disease in untreated patients or late treated with FH is significant (Figure 2). As the atherosclerotic burden is dependent on the degree and duration of exposure to raised LDL-cholesterol levels, early diagnosis and initiation of treatment is paramount. Current evidence suggests the majority of patients with FH remain undiagnosed until the detrimental consequences of atherosclerosis have been established and this is mainly due to the lack of awareness among physicians and the general public.[(1)] Early diagnosis followed by treatment with intense lipid lowering agents could prevent occurrence of cardiovascular events by reducing the accumulative LDL-C. Recent data suggested the prevalence of FH among acute coronary syndrome (ACS) patients in Europe is between 1.6% and 8.3% when using clinical algorithms.[(2)] The prevalence of phenotypical FH in patients with premature coronary artery disease has a wide range amongst different ethnic groups **(Table-1)**.[(4-13)] Studies in Europe reported up to 8% of adults with acute coronary syndromes have been shown to have clinical criteria compatible with FH, this prevalence is extensively higher (>10 times) than the prevalence of FH in the general population.[(14)]

Recent studies reported higher rates of cardiac related complications such as recurrent ACS (over 2-fold) within the first year of the index event [(15)], higher occurrence of multi-vessel disease, lower left ventricular ejection fraction, higher proportion of cardiogenic shock or class IV heart failure and

lower percentage of achieved recommended target level of LDL-C despite statin therapy in one year follow up, among patients with FH post ACS.[5]

Table-1 Prevalence of FH in patients with coronary artery disease [16]

Author	Year	Region	n	Age, y	Prevalence (%)	Criteria
Auckle et al	2017	China	498	<60	3.8	DLCN
Motensen et al	2016	Denmark	1381	<60	2.0/4.7	DLNC/SBR
Li et al	2016	China	1843	<60	3.9	DLCN
Pang et al.	2015	Australia	175	<60	14.3	DLCN
De Backer et al.	2015	Europe	2212	<60	15.4	DLCN
Yudi et al.	2012	Australia	201	<60	1.4	DLCN
Wiesbauer et al.	2009	Austria	302	<40	8	SBR
Bates et al.	2008	Australia	199	<60	2.1	DLCN
Rallidis et al.	2008	Greece	135	<35	19	SBR
Dorsch et al.	2001	UK	292	<60	12	SBR

DLCN*: Dutch Lipid Clinic Network;* ***Chol****: Cholesterol;* ***SBR****: Simon Broom Register*

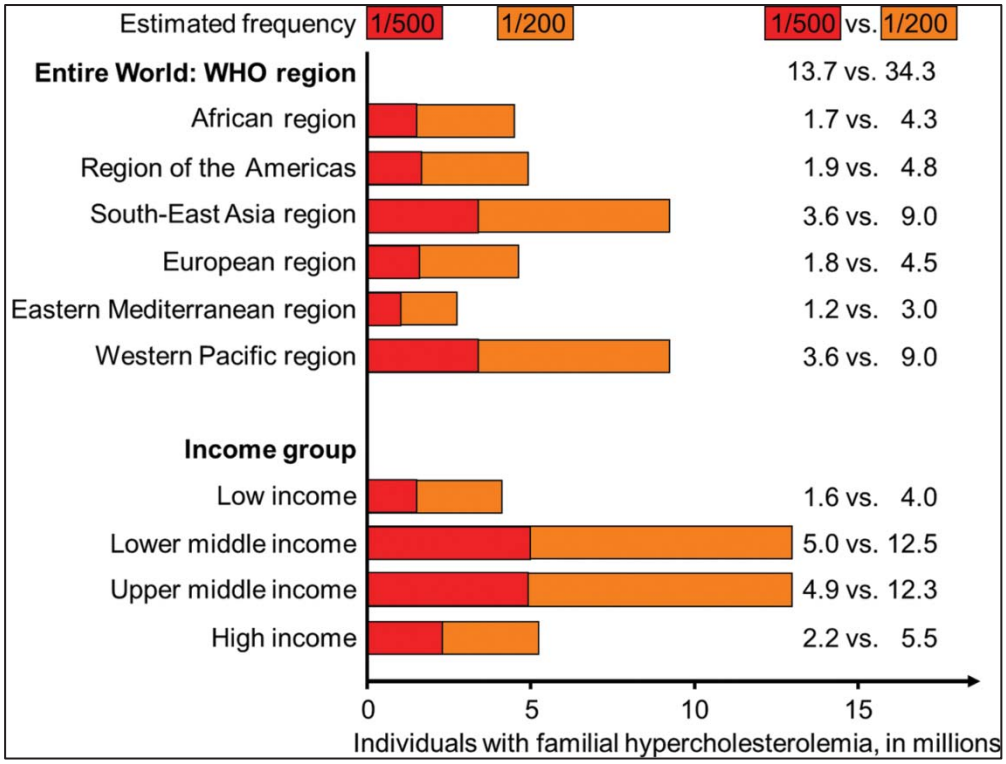


Figure 3- *Estimated frequency rate of FH in the world* [(1)]

Former studies also demonstrated patients with FH and ACS have over two times higher risk of recurrent cardiovascular events.[(17)] Early diagnosis is important among patients with ACS allowing prompt intense lipid-lowering treatment and further cascade screening of their first degree relative to prevent early cardiovascular events. The vast majority of individuals with FH in Australia are currently undiagnosed, and those who are diagnosed (~20%), are often inadequately treated.[(18)] Current evidence shows there is a substantial gap in diagnosis and treatment of patients with FH, the reasons vary from insufficient documentation in medical records to suboptimal treatment according to the national heart foundation target level recommendation.[(18, 19)]

B-Diagnosis of familial hypercholesterolemia

Early diagnosis of FH to prevent complications and achieving an effective treatment outcome is crucial (Figure-4).[(1, 20)] The diagnosis of FH is made on the basis of clinical history, physical examination and genetic testing. There are three gene mutations (APOB, LDLR and PCSK9) known

to cause autosomal dominant FH, however these can be found in only ~40% of patients with a clinical diagnosis of FH.[21] There is a heterogeneity in phenotypical expression of and response to therapy depending on the type of genetic mutation. Due to poor correlation between phenotypical findings and diagnosis of FH, several clinical diagnostic criteria have been developed to identify FH. Among the most widely used FH clinical criteria are the Dutch Lipid Clinic Network (DLCN) [22] and the Simon Broome (SB) Register Group in the United Kingdom.[23] A comparison study demonstrated DLCN has the highest sensitivity and MEDPED has the highest specificity for diagnosis of patients with FH.[24]

By knowing that all FH diagnostic and screening tolls are based on clinical signs, reviewing patients with possible FH by a physician who has special interest in clinical lipidology would be paramount.

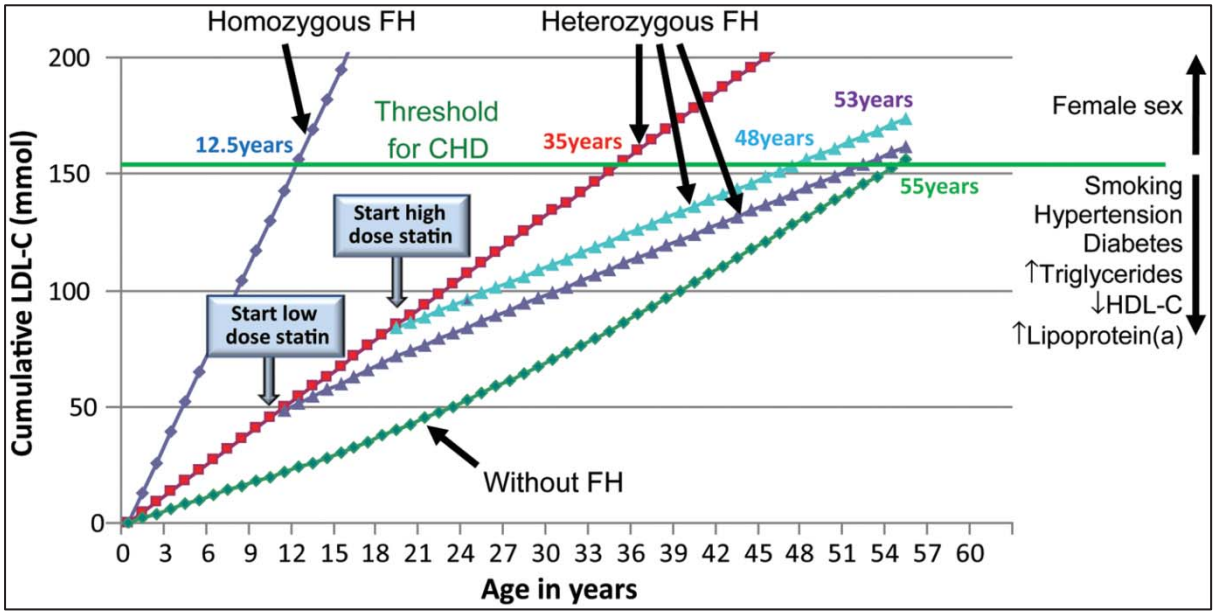


***Figure 4**- The relationship between cumulative LDL-C and age of CHD [1]*

C-Screening for FH

Prevention of premature cardiovascular disease by earlier diagnoses and treatment in patients with FH is crucial. FH is an autosomal dominant lipid disorder, therefore the probability that children and

siblings of patients with FH have the condition is high at 50%. There are a number of proposed strategies to screen for FH including universal screening [25], opportunistic screening of individuals with high risk of ASCVD and finally cascade testing.

Among them cascade screening in first degree relatives of patients with FH has been demonstrated to be cost effective and is recommended in several FH guidelines. Screening children for FH using cholesterol measurement between the age of 2 to 10 years is recommended as the optimal time.[1] Also, cost effectiveness of cascade screening by genetic testing, measuring cholesterol levels and appropriate treatment in prevention of CVD events has been demonstrated consistently in the UK.[26] Bell et al reported genetic cascade screening through a centralised service in an Australian settingis an effective and acceptable strategy for detecting FH.[27]

Awareness and knowledge of medical practitioners about diagnostic criteria of FH is the cornerstone of having an efficient screening system. For physicians to be aware of clinical signs and hallmarks of FH during daily practice is essential but they are simply missed as shown from former studies.[3] Bell et al. demonstrated the imperative role of community laboratory for opportunistic screening of patients with FH.[18]

D-Diagnosis of subclinical ASCVD in patients with FH

i. Intima media thickness

Carotid intima media thickness (CIMT) is approved as a surrogate marker of subclinical ASCVD and a predictor of events in the general population as well as patients with FH.[28, 29]

However, due to several caveats and lack of a standard protocol [30] the application of CIMT in routine risk stratification is controversial. The 2012 European Society of Cardiology recommends CIMT in patients with intermediate risk of cardiovascular disease (Class IIa; reasonable)[31],

whereas the 2013 American College of Cardiology/American Heart Association (ACC/AHA) does not recommend it (Class III; no benefit) in any clinical setting.[32]

ii. Multi-detector computed tomography coronary angiography

CT Coronary angiogram (CTCA) is a widely established modality to assess clinical and subclinical atherosclerotic disease. Particularly the use of calcium scoring as a strong predictor of cardiac events has been approved.[33] Only a few trials have studied plaque burden in FH patients used CTCA. Tada et al demonstrated on 101 individuals with heterozygous FH that plaque burden score is an independent risk factor for future cardiovascular events with a linear correlation.[34] They also concluded the development of coronary plaque initiate in earlier stage of life in patients with FH, 23 years of age in male and 34 in female individuals with FH.[34]

Coronary plaque burden and composition have also been assessed by CTCA comparing FH vs non-FH individuals with clinical symptoms, demonstrating greater atheroma burden and calcium score in FH. Equally studies assessing subclinical FH patients reported high plaque burden despite intense statin therapy.[35, 36] The value of coronary calcium score in prediction of significant CAD and future cardiovascular risk has also been endorsed in patients with FH.[36] However, the value of calcium score in FH patients has remained controversial due to insufficient available data to be definitive.

Epicardial fat is a novel parameter that can be quantified by cardiac CT in patients with FH and it is independently associated with the presence and severity of subclinical atherosclerosis burden.[37]

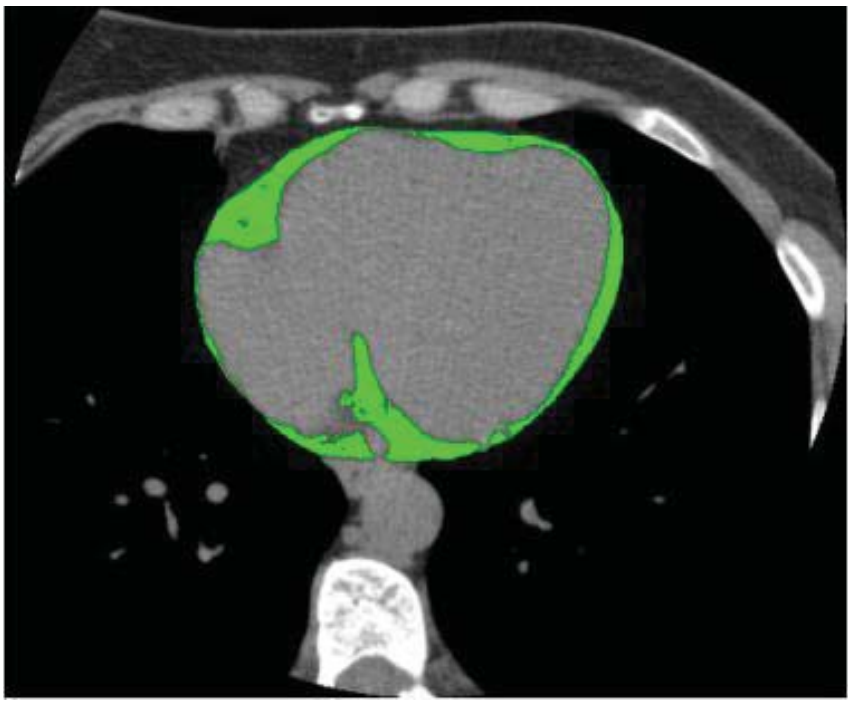


Figure 4- *Epicardial fat measured by semi-automated method via cardiac CT*

E-Cardiovascular risk stratification in FH

FH is associated with premature ASCVD and increased atheroma burden in affected individuals. There is a heterogeneity among patients with FH in terms of the onset of atherosclerotic disease and severity of atheroma burden. Interestingly, this heterogeneity has also been reported between index and cascade screened cases.[38] Conventional cardiac risk factors including diabetes, smoking, hypertension, in addition to age, gender and low high density lipoprotein (HDL), to some extent can explain this diversity.[37]

Furthermore, there are a few implicit elements which can considerably impact on the course of atherosclerotic disease in patients with FH. Patients with monogenic FH have higher extent of cardiovascular events.[39] Lipoprotein (a) is an atherogenic LDL-C like particle in plasma consistently reported to be noticeably higher in patients with FH and associated with early onset

cardiovascular events, irrespective of LDL-C levels.[40] Also, tendon xanthoma is an independent predictor of atherosclerosis.[41]

E-Treatment of hypercholesterolemia in patients with FH

Treatment with LDL-C lowering agents will significantly improve all cardiovascular outcomes in patients with FH.[42] However, LDL-C level in the majority of patients with FH, even with high dose of statin monotherapy, remains elevated.[43] Therefore special attention should be taken in management of this high risk group.

F- Future direction in management of familial hypercholesterolemia

- Suboptimal knowledge and awareness of national guidelines, hereditability, prevalence and diagnostic features of FH among medical practitioners.[44]
- The absence of cardiovascular risk estimation tools for FH.
- Lack of a uniform screening program for FH in the community.
- A cost effective modality to screen patients with FH and subclinical ASCVD.
- There is insufficient data with limited methodology to demonstrate a strong association between FH and stroke events.[29]
- FH should be taken as a public health matter, a valuable strategy should be developed to fill the gap in diagnosis.
- Ongoing education and workshops to educate medical practitioners and introducing FH to curricular scheme in medical schools and college of physicians for systematic training.
- A family-based cascade screening program with the integrated primary care (PC) physicians and specialist lipid clinics.

References:

1. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease Consensus Statement of the European Atherosclerosis Society. European Heart Journal. 2013;34(45):3478-90.

2. Amor-Salamanca A, Castillo S, Gonzalez-Vioque E, Dominguez F, Quintana L, Lluís-Ganella C, et al. Genetically Confirmed Familial Hypercholesterolemia in Patients With Acute Coronary Syndrome. Journal of the American College of Cardiology. 2017;70(14):1732-40.

3. Schofield J, Kwok S, France M, Capps N, Eatough R, Yadav R, et al. Knowledge gaps in the management of familial hypercholesterolaemia. A UK based survey. Atherosclerosis. 2016;252:161-5.

4. Compensatory Enlargement of Human Atherosclerotic Coronary Arteries. New England Journal of Medicine. 1987;317(25):1604.

5. Auckle R, Su B, Li H, Xu S, Xie M, Song Y, et al. Familial hypercholesterolemia in Chinese patients with premature ST-segment-elevation myocardial infarction: Prevalence, lipid management and 1-year follow-up. PLoS One. 2017;12(10):e0186815.

6. De Backer G, Besseling J, Chapman J, Hovingh GK, Kastelein JJP, Kotseva K, et al. Prevalence and management of familial hypercholesterolaemia in coronary patients: An analysis of EUROASPIRE IV, a study of the European Society of Cardiology. Atherosclerosis. 2015;241(1):169-75.

7. Yudi M, Omera L, McCubbery N, Dick S, Jayasinghe R, Hamilton-Craig I. Suboptimal consideration and management of potential familial hypercholesterolaemia in patients with suspected premature coronary artery disease. Singapore medical journal. 2012;53(3):174-8.

8. Dorsch MF, Lawrance RA, Durham NP, Hall AS. Familial hypercholesterolaemia is underdiagnosed after AMI. BMJ. 2001;322(7278):111.

9. Wiesbauer F, Blessberger H, Azar D, Goliasch G, Wagner O, Gerhold L, et al. Familial-combined hyperlipidaemia in very young myocardial infarction survivors (≤40 years of age). European Heart Journal. 2009;30(9):1073-9.

10. Bates TR, Burnett JR, Bockxmeer FMv, Hamilton S, Arnolda L, Watts GF. Detection of Familial Hypercholesterolaemia: A Major Treatment Gap in Preventative Cardiology. Heart, Lung and Circulation. 2008;17(5):411-3.

11. Loukianos SR, John L, Demosthenes P, Katerina F, Christoforos K, Thomas A, et al. Long-term prognostic factors of young patients (≤ 35 years) having acute myocardial infarction: the detrimental role of continuation of smoking. European Journal of Cardiovascular Prevention & Rehabilitation. 2008;15(5):567-71.

12. Li S, Zhang Y, Zhu CG, Guo YL, Wu NQ, Gao Y, et al. Identification of familial hypercholesterolemia in patients with myocardial infarction: A Chinese cohort study. Journal of clinical lipidology. 2016;10(6):1344-52.

13. Mortensen MB, Kulenovic I, Klausen IC, Falk E. Familial hypercholesterolemia among unselected contemporary patients presenting with first myocardial infarction: Prevalence, risk factor burden, and impact on age at presentation. Journal of clinical lipidology. 2016;10(5):1145-52.e1.

14. De Backer G, Besseling J, Chapman J, Hovingh GK, Kastelein JJ, Kotseva K, et al. Prevalence and management of familial hypercholesterolaemia in coronary patients: An analysis of EUROASPIRE IV, a study of the European Society of Cardiology. Atherosclerosis. 2015;241(1):169-75.

15. Nanchen D, Gencer B, Muller O, Auer R, Aghlmandi S, Heg D, et al. Prognosis of Patients With Familial Hypercholesterolemia After Acute Coronary SyndromesClinical Perspective. Circulation. 2016;134(10):698-709.

16. Pang J, Poulter EB, Bell DA, Bates TR, Jefferson V-L, Hillis GS, et al. Frequency of familial hypercholesterolemia in patients with early-onset coronary artery disease admitted to a coronary care unit. Journal of clinical lipidology. 2015;9(5):703-8.

17. Nanchen D, Gencercare unit. Journal of clinical lipidology. 2015;9(5):703-8. B, Muller O, Auer R, Aghlmandi S, Heg D, et al. Prognosis of Patients With Familial Hypercholesterolemia After Acute Coronary Syndromes. Circulation. 2016;134(10):698-709.

18. Bell DA, Hooper AJ, Bender R, McMahon J, Edwards G, van Bockxmeer FM, et al. Opportunistic screening for familial hypercholesterolaemia via a community laboratory. Annals of clinical biochemistry. 2012;49(Pt 6):534-7.

19. Bates TR, Burnett JR, Bockxmeer FMv, Hamilton S, Arnolda L, Watts GF. Detection of Familial Hypercholesterolaemia: A Major Treatment Gap in Preventative Cardiology. Heart, Lung and Circulation.17(5):411-3.

20. Goldberg AC, Robinson JG, Cromwell WC, Ross JL, Ziajka PE. Future issues, public policy, and public awareness of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. Journal of clinical lipidology. 2011;5(3 Suppl):S46-51.

21. Sharifi M, Futema M, Nair D, Humphries SE. Genetic Architecture of Familial Hypercholesterolaemia. Current Cardiology Reports. 2017;19(5):44.

22. Civeira F. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004;173(1):55-68.

23. Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ (Clinical research ed). 1991;303(6807):893-6.

24. Shin DG, Han SM, Kim DI, Rhee M-Y, Lee B-K, Ahn YK, et al. Clinical features of familial hypercholesterolemia in Korea: Predictors of pathogenic mutations and coronary artery disease 2013; A study supported by the Korean Society of Lipidology and Atherosclerosis. Atherosclerosis.243(1):53-8.

25. Klancar G, Groselj U, Kovac J, Bratanic N, Bratina N, Trebusak Podkrajsek K, et al. Universal Screening for Familial Hypercholesterolemia in Children. Journal of the American College of Cardiology. 2015;66(11):1250-7.

26. Lázaro P, Pérez de Isla L, Watts GF, Alonso R, Norman R, Muñiz O, et al. Cost-effectiveness of a cascade screening program for the early detection of familial hypercholesterolemia. Journal of clinical lipidology. 2017;11(1):260-71.

27. Bell DA, Pang J, Burrows S, Bates TR, van Bockxmeer FM, Hooper AJ, et al. Effectiveness of genetic cascade screening for familial hypercholesterolaemia using a centrally co-ordinated clinical service: an Australian experience. Atherosclerosis. 2015;239(1):93-100.

28. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Witteman JC, Grobbee DE. [Association between the intima-media thickness of the common carotid and subsequent cardiovascular events in subjects, 55 years and older, in the Rotterdam study (ERGO)]. Nederlands tijdschrift voor geneeskunde. 1998;142(19):1100-3.

29. Hutter CM, Austin MA, Humphries SE. Familial Hypercholesterolemia, Peripheral Arterial Disease, and Stroke: A HuGE Minireview. American journal of epidemiology. 2004;160(5):430-5.

30. Baroncini LAV, de Castro Sylvestre L, Filho RP. Carotid intima-media thickness and carotid plaque represent different adaptive responses to traditional cardiovascular risk factors. IJC Heart & Vasculature. 2015;9:48-51.

31. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2012;33(13):1635-701.

32. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Sr., Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology. 2014;63(25 Pt B):2935-59.

33. Weber LA, Cheezum MK, Reese JM, Lane AB, Haley RD, Lutz MW, et al. Cardiovascular Imaging for the Primary Prevention of Atherosclerotic Cardiovascular Disease Events. Current Cardiovascular Imaging Reports. 2015;8(9):36.

34. Tada H, Kawashiri MA, Okada H, Teramoto R, Konno T, Yoshimuta T, et al. Assessment of coronary atherosclerosis in patients with familial hypercholesterolemia by coronary computed tomography angiography. The American journal of cardiology. 2015;115(6):724-9.

35. Neefjes LA, Ten Kate GJ, Rossi A, Galema-Boers AJ, Langendonk JG, Weustink AC, et al. CT coronary plaque burden in asymptomatic patients with familial hypercholesterolaemia. Heart (British Cardiac Society). 2011;97(14):1151-7.

36. Miname MH, Ribeiro MS, 2nd, Parga Filho J, Avila LF, Bortolotto LA, Martinez LR, et al. Evaluation of subclinical atherosclerosis by computed tomography coronary angiography and its association with risk factors in familial hypercholesterolemia. Atherosclerosis. 2010;213(2):486-91.

37. Mangili LC, Mangili OC, Bittencourt MS, Miname MH, Harada PH, Lima LM, et al. Epicardial fat is associated with severity of subclinical coronary atherosclerosis in familial hypercholesterolemia. Atherosclerosis. 2016;254:73-7.

38. Silva PRS, Jannes CE, Marsiglia JDC, Krieger JE, Santos RD, Pereira AC. Predictors of cardiovascular events after one year of molecular screening for Familial hypercholesterolemia. Atherosclerosis.250:144-50.

39. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. Heart (British Cardiac Society). 2016.

40. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. European Heart Journal. 2010;31(23):2844-53.

41. Mangili LC, Miname MH, Silva PRS, Bittencourt MS, Rocha VZ, Mangili OC, et al. Achilles tendon xanthomas are associated with the presence and burden of subclinical coronary atherosclerosis in heterozygous familial hypercholesterolemia: A pilot study. Atherosclerosis. 2017;263:393-7.

42. Huxley RR, Hawkins MH, Humphries SE, Karpe F, Neil HAW. Risk of Fatal Stroke in Patients With Treated Familial Hypercholesterolemia. A Prospective Registry Study. 2003;34(1):22-5.

43. Reiner Ž. Management of patients with familial hypercholesterolaemia. Nature Reviews Cardiology. 2015;12:565.

RESEARCH AIMS

The aim of this theses is to first outline current evidence of awareness, prevalence, prognosis, and characteristics of patients with FH **(Chapter 2)** and subsequently to compare them to our data from patients with premature ACS admitted to coronary care units **(Chapter 3).** In order to diagnose patients with FH in CCU, medical staff managing ACS need to have an adequate knowledge and understanding about FH, therefore we performed a multicentre survey to evaluate knowledge and practice in coronary care **(Chapter 3)**. We also explored the barrier and bridges to improve screening and care of patients with FH in tertiary hospital by auditing and case studies **(Chapter 3)**. Consequently, we used state-of- the- art tools to evaluate elements which may increase the risk of cardiovascular disease among patients with FH to optimise risk stratification in this high risk group. Emerging evidence indicates peri-coronary adipose tissue attenuation (PCAT) is an important parameter to assess vasucular inflammation as one of the predictor of future events therefore we performed PCAT analysis via CTCA in FH patients with coronary artery disease and without disease **(Chapter 4)**. To understand the methodology of evaluating coronary arteries two systematic reviews and meta-analysis were performed assessing plaque regression and progression in response to intense lipid lowering treatment in **Chapter 2**. Finally, we use of a simplified oral fat tolerance test to evaluate post prandial lipaemia as an additional risk for cardiovascular disease in patients with FH **(Chapter 5)**.

Chapter 2

Methodology and Systematic Reviews

Preface

In this chapter we have reviewed current and immerging lipid lowering management for patients with FH and high risk cardiovascular disease. We have also outlined the role of different cardiac investigation tools and techniques such as intravascular ultrasound (IVUS), Quantitative coronary angiogram (QCA) and mean luminal diameter (MLD) to assess impact of lipid lowering management on plaque progression, cardiovascular events and ultimate prognosis. This chapter contains three original research papers in review, systematic review and meta-analysis format.

Chapter 2 A

New Era in the Management of Atherosclerosis; Current and Emerging Novel Lipid Modulators

New Era in the Management of Atherosclerosis; Current and Emerging Novel Lipid Modulators

Sam Mirzaee[1], James CG Doery[2], Arthur Nasis[1,] Dennis TL Wong[1], James D Cameron[1].

[1] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia
[2] Monash Health Pathology, Monash Health, Melbourne, Australia

Correspondence:

Dr Sam Mirzaee

MonashHeart, Monash Health

246 Clayton Rd, Clayton, 3168, Victoria

Email: Sam.Mirzaee@monashhealth.org

Abbreviations

ACS: Acute coronary syndrome

AIDS: Acquired immunodeficiency syndrome

Apolipoprotein (AI): apo-A-I

Apolipoprotein (B): apo(B)

Apolipoprotein (E): apo(E)

ASCVD: Atherosclerotic cardiovascular disease

BA: Bempedonic acid

BBR: Berberine

CAD: Coronary artery disease

CETP: Cholesteryl ester transfer protein

CVD: Cardiovascular disease

FDA: Food and Drug Administration (USA)

FH: Familial hypercholesterolemia

FCH: Familial combined hyperlipidaemia

HoFH: Homozygous familial hypercholesterolaemia

HMG-CoA: 3-hydroxyl-3-methyl-glutaryl-Coenzyme-A

IV: Intravenous

hCRP: high sensitivity C-reactive protein

HDL-C: High-density lipoprotein cholesterol

LDL-C: Low-density lipoprotein cholesterol

LDL-R: Low-density lipoprotein receptor

LP(a): Lipoprotein (a)

MACE: Major adverse cardiovascular events

MTP: Microsomal Triglyceride Transfer Protein

NA: Nicotinic acid

PPAR: Peroxisome proliferator-activated receptor

PCSK-9: Proprotein Convertase Subtilisin/Kexin type 9

SREBP2: Sterol-responsive element-binding protein 2

TGRL: Triglyceride-rich lipoproteins

QW: Weekly

Q2W: Biweekly

Q4W: Four-weekly

QM: Monthly

SC: Subcutaneous

ABSTRACT

Hypercholesterolemia remains one of the most important modifiable risk factors for coronary artery disease as the leading cause of death in Australia. Until recently, hydroxyl-methyl-glutaryl-Coenzyme-A (HMG-CoA) reductase inhibitors were the most effective low-density lipoprotein cholesterol (LDL-C) lowering therapy shown to offer significant cardiovascular and mortality benefits. Despite effective initial treatment strategies, a large body of epidemiological evidence indicates a significant proportion of patients with cardiovascular diseases fail to achieve recommended lipid targets or adequate cardio vascular prevention for various reasons. Among them are those with hereditary lipid disorders and those intolerant to statins. We outline the latest advances in clinical lipidology with lipid-lowering strategies, the physiology, and function of current and imminent novel lipid-lowering therapies. The impact of these novel treatments including proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors on cardiovascular outcomes, coronary atheroma burden, and overall mortality is promising; however, the cost-effectiveness, their target population and the precise mechanism of action on atherosclerosis remain of interest. Emerging novel anti lipid agents in conjunction with progress in genomic testing is providing a basis of personalised medicine in the management of atherosclerosis.

Keywords: Novel lipid-lowering, cardiovascular disease, PCSK-9 inhibitors, atherosclerosis

INTRODUCTION

Cardiovascular disease (CVD) claims the lives of 17.7 million people every year, accounting for approximately 30% of all global deaths and remaining the leading cause of mortality in developed countries such as Australia (1, 2). Hyperlipidemia is an established major modifiable cardiovascular risk factor, and the degree of CVD depends on the etiology and the exact form of elevation in lipid components (3). The UK Simon Broome Familial Hyperlipidaemia Register has shown that the cumulative risk of either a premature fatal or non-fatal cardiac event is significantly higher in untreated patients with familial hypercholesterolaemia (FH) (4, 5).

Until recently, hydroxyl-3-methyl-glutaryl-Coenzyme-A (HMG-CoA) reductase inhibitors or statins have been the most efficient LDL-C lowering agent in the treatment of hypercholesterolemia (6, 7). Statins act primarily on the 3-hydroxyl-3-methyl-glutaryl-Coenzyme-A receptor through competitive inhibition which subsequently reduces cholesterol synthesis (8). Statin therapy leads to an improvement in both primary and secondary atherosclerotic cardiovascular events (9). In statin-resistant or intolerant patients, current guidelines recommend ezetimibe (Niemann-Pick C1-like protein 1 (NPC1L1) inhibitor for reducing intestinal cholesterol absorption); niacin (nicotinic acid) or bile acid sequestrants as second-line therapies (10).

Over the last three decades, different strategies have also been applied to lower low-density lipoprotein cholesterol (LDL-C) and associated cardiovascular consequences. These included surgical intervention by partial ileal bypass (POSCH trial) (11), lipid apheresis (12) and a simple administration of nutraceutical medicines; whereas, their efficacy in reduction of CVD events remains controversial. In many patients, however, the risk of a recurrent atherosclerotic cardiovascular disease (ASCVD) event remains high despite reaching proposed LDL-C target and administration of maximally tolerated disease of statins. Therefore, recent international lipid guidelines have included additional recommendations for adequate ASCVD prevention.

New treatment targets such as non-high density lipoprotein (Non-HDL) and triglyceride (TG) level (as a class-I) **(Figure 1)**, in conjunction with an effective diet, lifestyle modification, treatment of coexisting cardiac risk factors, non-fasting lipid testing and finally introduction of novel anti-lipid agents are the most prominent updates (6, 13). Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are an expensive novel class of LDL-C lowering agents with significant LDL-C lowering properties and acceptable cardiovascular and mortality benefits that are being increasingly utilised in managing high risk hyperlipidaemia listed as Class II-b recommendation **(Figure 2)** (13). This review evaluates the current evidence regarding the clinical utility of lipid-lowering therapies and provides further insights into the cardiovascular benefits and their safety.

LIPOPROTEINS AND GENETIC MUTATIONS IN ATHEROSCLEROSIS

LDL-C is the most studied lipoprotein associated with CVD and atherosclerosis (14). Mendelian randomisation studies have reinforced the imperative role of early and sustained lowering of LDL-C to prevent the development of atherosclerotic disease (15). PCSK-9 is a recently discovered modulator in the homeostasis of LDL-C (16) binding to LDL receptors (LDL-R) which prevent internalisation of the receptor and subsequent degradation. Therefore, PCSK-9 level is negatively correlated with the number of available LDL-R on the surface of hepatocytes (**Figure 1**) (17, 18); subsequently down-regulation of LDL-R leads to higher circulating LDL-C.

The discovery of PCSK-9 mutation in families with autosomal-dominant FH was a fascinating medical breakthrough (19). It has consequently been demonstrated that PCSK-9 gene variation result in varying levels of circulating LDL-C. Gain-of-function PCSK-9 mutation results in lower levels of LDL receptors (LDL-R) and therefore higher circulating LDL-C. Inversely, a loss-of-function mutation of PCSK-9 produces a lower level of LDL-C and apolipoprotein B (apo-B), corresponding to a marked reduction in coronary events (20).

Apo-B is another atherogenic lipoprotein which contains very low density lipoproteins (VLDL) and its derivatives plus LDL-C. Therefore it is a reasonable therapeutic target for reducing the concentration of hypercholesterolemia, particularly those with genetic LDL-R defect. Previously, the role of TG and TG-rich lipoprotein (TGRL) in atherosclerosis was contentious, though further epidemiological data demonstrated robust associations with ASCVD perhaps through cascade of inflammation and reverse cholesterol transportation (21).

Lipoprotein (a) or Lp(a) is an LDL-C-like lipoprotein in plasma, resembling the functionality of plasminogen protein. It is a highly atherothrombogenic lipoprotein; under strong genetic control and leading to an increased risk of ASCVD events and aortic valve calcification (22). Current guidelines recommend screening of Lp(a) solely in patients with rapid progressive coronary artery disease (CAD) and those with either a high CV risk or a strong family history of early onset atherothrombotic disease (22).

High density lipoprotein cholesterol (HDL-C) is a small and dense lipoprotein particle, inversely associated with the risk for the development of ASCVD. The protective mechanism of HDL-C is pleotropic and complex, exhausting its beneficial effects through an antioxidant effects and interactions with TGRLs resulting in improvement of endothelial function, coagulation parameters, and inflammation (23). Several genotypes have been associated with CVD and hyperlipidaemia among them apolipoprotein-E (apoE) and of genes associated with FH (LDLRs, apo-B and PCSK9) are more common and should be considered in certain high risk patients. ApoE (apoE2, apoE3 and apoE4) genotyping is indicated in cases with severe combined hyperlipidaemia and is mainly used for the diagnosis of dysbetalipoproteinaemia (apoE2 homozygosity) (11).

NOVEL BIOMOLECULAR TARGETS FOR PLASMA LIPID REGULATION

1. LDL-C MODULATORS

1.1 PCSK-9 Inhibitors

PCSK-9 inhibitors target PCSK-9 at varying pathways with the aim of an eventual increases in the number of functional LDL-R on cell surfaces resulting in a reduction of LDL-C. Sterol-responsive element-binding protein 2 (SREBP2) is a regulator of transcription of PCSK-9, which also plays a role as a transcription factor for both LDL-R and HMG-CoA reductase (24). Production of HMG-CoA reductase and LDL-R is intensified by reduced intra-hepatocyte cholesterol levels, which leads to the increased biosynthesis and uptake of circulating LDL-C particles into hepatocytes (25). These regulators are a potential pharmacological target to treat hyperlipidaemia.

i. Monoclonal antibodies

Three monoclonal antibodies (mAb) have been developed to selectively target PCSK-9; evolocumab, alirocumab and bococizumab. Another monoclonal antibody, LY3015014 has undergone phase-II trials but has not reached further development (26). Evolocumab and alirocumab are currently available fully human mAbs and have been approved for use in high risk subjects such as FH, progressive coronary artery disease and statin intolerance. Bococizumab is a humanised monoclonal antibody the production of which has been terminated due to developing antidrug antibodies. Meta-analysis of bococizumab data; six Studies of PCSK-9 Inhibition and the Reduction of Vascular Events (SPIRE) trials demonstrated most patients had marked reduction of LDL-C in the early course of treatment (12 weeks); at 52 weeks, however, the efficacy is substantially diminished due to the development of neutralising drug antibodies (27).

The GLAGOV (Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound) trial studied the effect of evolocumab on progression of coronary disease revealed that over 76 weeks of treatment with statins, the addition of evolocumab in subjects with

the angiographic coronary disease caused a 0.95% reduction in percent atheroma volume (PAV) (28). The FOURIER trial also consistently demonstrated that combined evolocumab and statin therapy, after a median of 26 months follow up significantly reduced the risk of cardiovascular events without significant safety concerns (29). Recently, ODYSSEY OUTCOMES demonstrated significant improvement in composite of death from ASCVD events in patients with acute coronary syndrome using alirocumab in addition to high intensity statin (30).

ii. Small interfering RNA (siRNA)

Antisense oligonucleotides (ASOs) are another novel therapy that modulates expression of specific genes by binding to complementary messenger RNA (mRNA). Inclisiran is an example of ASOs, a siRNA, regulates gene expression of PCSK-9 by silencing mRNA which leads to a modest reduction in plasma LDL-C levels (14). Recently, a phase two multi-centre randomised control trial (ORION-1) investigated the role of inclisiran in patients at high risk of cardiovascular disease and elevated plasma LDL-C (above 1.8 mmol/L) on a maximum tolerated dose of statin. Participants were randomised to one of six different subcutaneous dosing regimens of PCSK-9si or placebo. This phase-II trial confirmed the safety of inclisiran and demonstrated that the optimal dosing regimen was 300 mg inclisiran at day one and 90, leading to as much as a 52.5% reduction of plasma LDL-C (31). The ORION-3 and ORION-4 trial will investigate its impact on cardiovascular outcomes and trial a biannual regimen of inclisiran.

iii. Active immunisation

Anti-PCSK-9 vaccine (AT04A) has recently been studied in a translational animal model to evaluate the modulation of circulating cholesterol level and atheroma. The study demonstrated significant improvement in plasma lipid profiles followed by a beneficial effect on systemic, vascular inflammation, and atherosclerotic lesions in the aorta (32). This study has suggested the possibility of a potential cost effective and robust long-term management of elevated circulating LDL-C level along with cardiovascular benefits.

1.2 Liver-specific adenosine triphosphate citrate lyase inhibitor

Bempedonic acid (BA) is a promising novel LDL-C lowering therapy proposed for patients with statin intolerance. It acts by upregulation of LDL-R through inhibition of adenosine triphosphate (ATP) citrate lyase; an enzyme located upstream from HMG-CoA reductase pathway, effects on fatty acid biosynthesis (**Figure 1**). Efficacy and safety of BA added to ezetimibe in statin-intolerant patients studied in a randomised, placebo-controlled study showed not only effective LDL-C reduction and LDL-R modulation, but also improvement in inflammation, endothelial dysfunction, and hypertension (33). ESPERION phase-III clinical trial reported BA appeared to be safe, well tolerated and provide 18% additional LDL-C lowering and 19% reduction of high sensitivity C reactive protein (hCRP) in patients on optimal statin therapy (34, 35) . The CLEAR outcome trial is an event-driven study currently assessing the effects of BA on the occurrence of major adverse cardiovascular events (MACE) in statin intolerant patients.

1.3 Novel Statins

Pitavastatin is a relatively novel statin which is primarily used in patients with acquired immunodeficiency syndrome (AIDS) on antiviral medications. Unlike other statins, it is predominantly metabolised by glucuronidation and only minimally by CYP450. Therefore it has minimal interactions with anti-retroviral medications. The INTREPID trail has studied the efficacy and safety of pitavastain compared to pravastatin in acquired immunodeficiency syndrome (AIDS) patients demonstrating a superior impact of pitavastatin on LDL-C lowering, markers of immune activations and arterial inflammation(23). REPRIEVE is another outcome trial similarly testing whether pitavastatin, can prevent vascular events over time among human immunodeficiency virus (HIV) infected individuals who do not have known CVD (23).

1.4 New nutraceutical forms

A recent study showed a compound berberine (BBR) inhibits PCSK-9 transcription leading to lower circulating PCSK-9 level (36), though the further cardiovascular benefits of BBR remains unclear. A recent review paper studied the effectiveness of nutraceutical LDL-C lowering agents namely Red Yeast Rice (RYR), plant sterols and BBR suggesting they should be used strategically and in combination with pharmacotherapy in those with modest elevation of LDL-C ; however their potential adverse events and further cardiovascular benefits remain to be established (37).

2. APOLIPOPROTEIN-B 100 INHIBITORS

ApoB-100 is the main lipoprotein component of LDL-C, which its dysfunction may involved in a range of metabolic disorders, such as atherogenesis and hypercholesterolemia.

i. **Mipomersen**

Mipomersen is an FDA-approved antisense oligonucleotide therapy which is administered by subcutaneous injection. It blocks gene translation by targeting apolipoprotein B-100 mRNA. Administration of mipomersen in patients with homozygous and severe heterozygous FH will cause an additional reduction in LDL-C of 25–37% from baseline. Furthermore, mipomersen is also a promising therapy in the management of elevated Lp(a) by causing a 20-30% reduction from baseline in patients with FH.

The significance of an increase in liver fat and elevated transaminase as a potential adverse events remains to be established (38). Long-term safety and efficacy of mipomersen administered up to 104 weeks, in patients with FH demonstrated persistent global reductions of lipoproteins with no major safety concerns. However, given injection site reactions and flu-like symptoms have been reported in the MICA-study, mipomersen is currently restricted to patients with FH and refractory elevated LDL-C despite lipoprotein apheresis (39). A post hoc analysis of recent trials showed that the long-term use of mipomersen may reduce MACE in FH patients (40) but studies exclusively assessing cardiovascular events as a primary endpoint are warranted.

3. HDL MODULATING AGENTS

There is a growing body of evidence demonstrating that HDL-C level is atheroprotective and inversely associated with cardiovascular outcome (41). Every 1 mg/dl (0.03 mmol/L) increase in plasma HDL-C is associated with a 2-3% reduction in the risk of developing CAD (42). This has opened a novel potential avenue in the management of cardiovascular disease by seeking modification in HDL-C metabolism (43).

i. Cholesteryl ester transfer protein (CETP) inhibitors

CETP converts cholesteryl esters from HDL to apoB-containing lipoproteins and increases HDL-C with potential atherosclerosis reduction. The ILLUMINATE study, a randomised controlled trial (RCT) on patients with high risk of CVD, using torcetrapib (the first CETP inhibitor) was prematurely halted due to an unexpected significant rise in mortality and morbidity in the treatment group (44). Subsequently, further CETP inhibitors such as dalcetrapib and evacetrapib had their trials prematurely aborted due to poor efficacy signals (45, 46). The safety of CETP inhibitors remains controversial as it appeared that there is a "U-shaped" association between HDL-C and all-cause mortality (43). A recent RCT using anacetrapib in addition to statin in subjects with CVD demonstrated a lower incidence of MACE compared with placebo with a promising safety profile (47).

ii. HDL mimetics

Apolipoprotein AI (apo-A-I) is the key protein component in HDL-C with multiple cardioprotective properties which improves reverse cholesterol transport (48). HDL mimetics are believed to increase cholesterol efflux, subsequently reduce atherosclerotic plaque volume and improve cardiovascular outcome by enhancing reverse cholesterol transport (**Figure 1**). The MILANO-PILOT trial assessed the efficacy of 5 weeks infusion of another HDL mimetic containing ApoA-IMilano (MDCO-216) in comparison with a placebo in 126 patients with acute coronary syndrome and angiographic coronary artery disease of 20-50% stenosis. This study evaluated efficacy and tolerability of MILANO-PILOT in addition to evaluation of the course of coronary atheroma as measured by intravascular ultrasound (IVUS). No significant benefit in reduction of total atheroma volume was demonstrated despite a satisfactory safety profile in these studies (48, 49).

iii. Nanomedicine

Nanomedicine is another evolving interdisciplinary field in cardiovascular medicine. Utilizing reconstituted HDL (rHDL) on nanocarriers to transfer therapeutic particles to intimal macrophage located in atherosclerotic plaques is another potential for management of atherosclerotic disease. This technology not only allows explicit delivery of anti-atherogenic agents into intimal macrophage (e.g. statin loaded r-HDL), also assisting reverse cholesterol transport from atheroma (50, 51).

4. LIPOPROTEIN (a) MODULATORS

Although current guidelines recommend plasma Lp(a) testing in high-risk patients, there is no consensus regarding the best treatment option in patients with high plasma Lp(a) levels (52, 53). Lp(a) apheresis has been a possible approach in patients with elevated Lp(a) and severe CVD, despite the paucity of prospective RCTs (54).

i. Nicotinic Acid Analogues

Acipimox, an analogue of nicotinic acid (NA), a lipoprotein modulator with fewer unwanted effects, inducing profound changes in various lipid and lipoprotein levels in plasma namely Lp(a) by up to 30% (49). The long term benefit of acipimax in obesity by improving lipid and glucose homeostasis has been demonstrated (55, 56). Moreover, there is no convincing data to show the cardiovascular benefits of using extended release NA/laropirant and acipimox (57, 58).

ii. *IONIS-APO (a) Rx*

This is another ASOs therapy with Lp(a) RNA-targeted mechanism which induces a lowering of plasma Lp(a) levels. It was tested in 2016 in a phase 2 trial using multiple doses (10mg, 20 mg, or 40 mg) in an ascending dose design and was compared with a placebo in two cohorts of patients with elevated Lp(a) (125-437 nmol/L in cohort A; ≥438 nmol/L in cohort B). The reported result suggested IONIS-APO(a)-LRx is a highly selective hepatocyte targeted medication and in the short term appears safe and well tolerated and importantly reducing Lp(a) levels by up to 92% (59).

5. MODULATORS of TRIGLYCERIDE RICH LIPOPROTEIN

High triglyceride is often associated with further lipoprotein disorders such as increased VLDL, apolipoprotein C-III (apo- C-III), raised total or small dense LDL-C particles, and reduced function of HDL-C, all of which seems to increase CVD (43, 60).

i. Microsomal Triglyceride Transfer Protein (MTP) Inhibitors

Lomitapide is an MTP inhibitor, FDA approved in 2013 and is restricted to use as an adjunct treatment for homozygous familial hypercholesterolemia (HoFH). Lomitapide acts in the liver with the remodelling of triglyceride-rich lipoproteins (TGRL) resulting in lower LDL-C. Since the mechanism of action is independent of LDL-R, they are suitable for HoFH with substantial LDLR deficiency. Lomitapide has been studied in a phase III trial in 29 subjects with HoFH on a maximum oral dose of 60 mg/day and this resulted in up to 40% reduction in plasma LDL-C from baseline (61). An increased risk of fatty liver and elevated liver transaminases has been reported, and its safety and effectiveness on cardiovascular morbidity and mortality remains to be established in patients with hypercholesterolemia without HoFH.

ii. Selective Peroxisome Proliferator-Activated Receptor (PPAR) Modulators

Pemafibrate, an effective novel selective PPAR modulator is involved in lipid metabolism and significantly reduces fasting TG levels by 45% in type 2 diabetes. The PROMINENT trial is currently studying cardiovascular outcomes of reduced triglyceride in this high risk subset (62).

iii. Omega 3 Fatty Acids and Derivatives

High-dose eicosapentaenoic acid (EPA) reduces triglyceride-rich lipoproteins without raising LDL-C. The REDUCE trial demonstrated effectiveness of icosapent ethyl (an EPA derivative) in reduction of ischemic events in statin-treated patients with high triglycerides (63, 64). Similarly, the STRENGTH trial assessing whether Epanova 4 g daily will decrease cardiovascular events in statin-treated high-risk patients with hypertriglyceridemia and low HDL-C levels (65).

iv. Modulator of Apolipoprotein C-III

ApoC-III is a primary modulator of TG and associated with CVD risk. Loss of function in ApoC-III seemed to be associated with in significant reduction in TG (40%) and further development of

cardiovascular disease (66). Volanesorsen is a second-generation ASO that can be specifically used to reduce levels of ApoC-III messenger RNA (mRNA) (67). Two recent Phase 3 studies APPROACH and COMPASS, including treatment with volanesorsen demonstrated safety with efficacy to decrease plasma TG levels by 77% and by 71%, respectively (68). This treatment option is suitable for patients with a very high plasma level of TG and at risk of CVD and acute pancreatitis.

v. Angiopoietin-like 3 inhibitors

Angiopoietin-like 3 (ANGPTL3) is a secreted protein expressed in the liver inhibiting lipoprotein lipase (LPL) which result in increased plasma levels of TG, LDL-C and HDL-C. ANGPTL3 modulator is another second generation ASOs to reduce ANGPTL3 and subsequently reduces elevated plasma TG and partially LDL-C. A recent study showing modulation of ANGPTL3 in both human and animal models was associated with significant reduction of all component of the lipid profile, predominantly up to 76% TG in addition to reduced ASCVD (69). Evinacumab, a fully human ANGPTL3-blocking antibody, was found to substantially lower LDL-C in adults with homozygous FH since functions independent to LDL receptor (70).

CHOICE OF THERAPY

Those with genetic hypercholesterolemia and statin intolerance are likely to gain the most benefits from novel lipid-lowering agents.

Genetic dyslipidaemias

a. **Familial hypercholesterolemia**: A number of trials have looked at the use of evolocumab in FH. Patients with heterozygous FH were studied in the RUTHERFORD-2 trial using evolocumab (140 mg Q2W and 420 mg QM) with a 60% LDL-C reduction observed at week 12 when compared with placebo (71). Likewise, the TESLA-B trial yielded a 31% reduction in LDL-C at 12 weeks when the efficacy of evolocumab was compared with the placebo in 50 stable patients with homozygous FH not on lipid apheresis (72). The ODYSSEY FH trial

evaluated the efficacy of alirocumab (75 – 150mg Q2W) in heterozygous FH patients, with at week 24, a 51-58% reduction in LDL-C was observed (73).

b. **Elevated Lipoprotein (a) with progressive ASCVD:** Increased level of Lp(a) is highly atherothrombogenic with higher risk of ASCVD and development of calcific aortic valve in genetically predisposed individuals. Current guidelines recommend screening of Lp(a) only in patients with rapid progressive CVD and those with either a high CV risk or a strong family history of early onset atherothrombotic disease (22). Lp(a) has been identified as an independent risk factor for cardiovascular disease but only a few effective Lp(a) modulating therapies are currently available. Although there is no consensus with regards to the best management for this high-risk group, the German reimbursement guideline covers lipid apheresis in patients with progressive ASCVD who have plasma Lp(a) > 60 mg/dl (74). Recent studies in patients treated with the monoclonal antibodies that target the PCSK-9 pathway observed a reduction of plasma Lp(a) levels to about 30% of baseline in a dose-dependent fashion (75). In terms of the need for an effective non-invasive treatment for modulating Lp(a) levels and its confirmed clinical significance in the development of ASCVD, PCSK-9 inhibitors may open a new avenue in the treatment of elevated Lp(a). However, cardiovascular benefits and a uniform definition of a normal plasma range of Lp(a) remain to be established.

c. **Familial combined hyperlipidaemia (FCH):** FCH is the most common inherited lipid disorder with elevated plasma apo-B and triglyceride resulting in development of CAD. Apo CIII is a main regulator of TG (76), therefore, there is a potential role of ApoCIII modulators in the management of refractory hereditary hypertriglyceridemia.

Statin intolerance

Patients with statin intolerance were specially excluded in most of the previously conducted trials in hypercholesterolemia, however the GAUSS-2 trial was conducted exclusively in the statin-intolerant

population when studying the efficacy of evolocumab (77). Statin intolerant patients were defined as being intolerant to at least two different statins and were randomised to either an evolocumab or ezetimibe arm. When compared with ezetimibe, evolocumab reduced plasma LDL-C levels by approximately 40% ($p < 0.001$). The ODYSSEY ALTERNATIVE study evaluated the efficacy of alirocumab in statin intolerant patients (unable to tolerate ≥2 statins, one at the lowest initiating dose) with the subjects randomised in a 2:2:1 fashion to alirocumab 75 mg Q2W, ezetimibe 10 mg daily and placebo (78). This study showed a 30%, further reduction in LDL-C with alirocumab in comparison to the ezetimibe arm. In both studies, a significant portion of participants (up to a third) on placebo reported muscle symptoms that highlight the role of nocebo effect in the statin-intolerant population.

DISCUSSION

Recent years have witnessed a rapid growth of new therapeutic targets for management of hyperlipidaemia. Several lipid-lowering agents have been developed to improve cardiovascular outcomes in high-risk individuals. The discovery of PCSK-9 led to the recent advancement in novel lipid-lowering therapies and facilitate of preventive medicine, particularly in high risk population and statin intolerants. Novel anti-lipid agents have also brought a new opportunity for patients who have been receiving invasive therapy (hereditary lipid disorders primarily HoFH) via lipid apheresis with anticipation of reducing the necessity for apheresis for many of these patients (79, 80). Cost effectiveness depending on overall risk of CVD, the age of target population and the cost of individual therapy remains the main challenge of using of novel therapies in routine practice (81).

Long-term safety data of novel lipid-lowering agents is scarce and requires further studies in addition to post-marketing analysis from real world practice. The emergence of novel medications has brought an opportunity to study the impact of very low level of LDL-C, even to the undetectable level, on cognitive function as this has been a significant concern in use of all lipid-lowering agents. Initial studies with PCSK-9 inhibitors showed a potential increased risk of neurocognitive defect, though

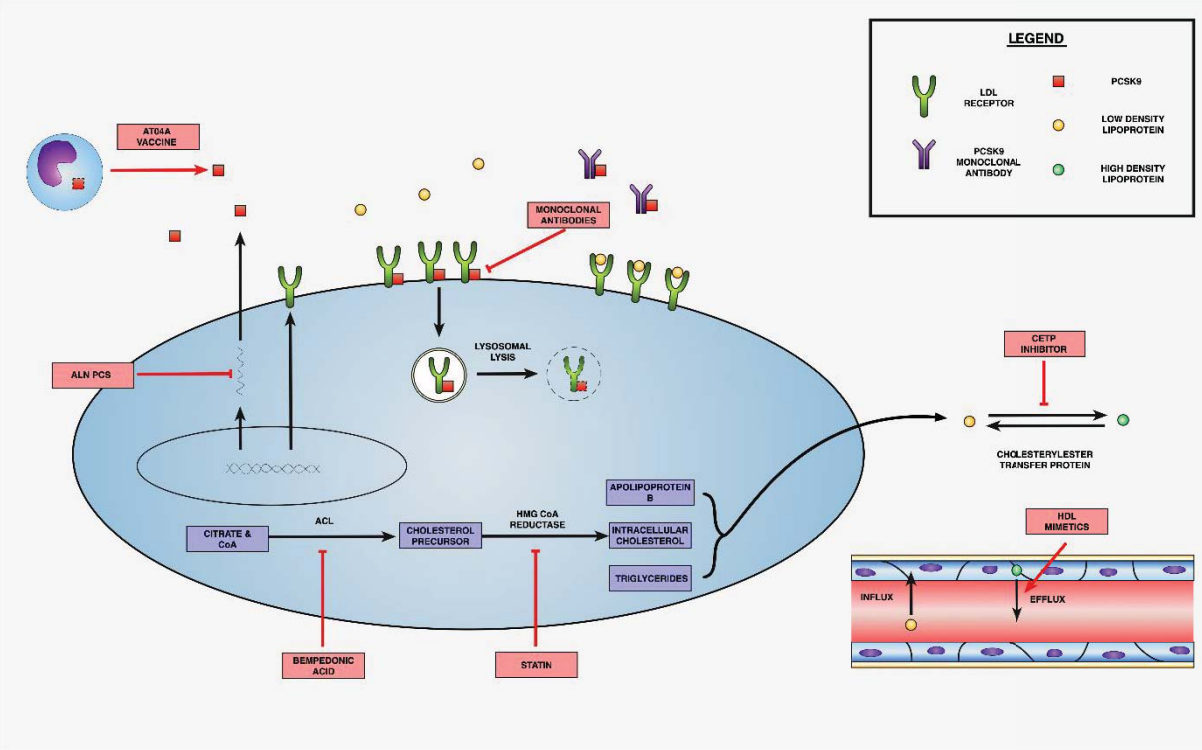
these were not formally evaluated. The EBBINGHAUS the first trial evaluated the effect of additional evolocumab to statin therapy on cognitive function using the Cambridge Neuropsychological Test Automated Battery. Reassuringly, this study demonstrated that evolocumab did not impact on either subjective reports or objective assessment of spatial working memory strategy (82).

A better understanding of the role of genetics in hyperlipidaemia disorders is the key as it can help diagnose hereditary disorders and aid in determining the prognosis and in tailoring management through target gene therapy. Gene testing may complement the investigation of lipid disorders in high risk population to guide clinicians to select the appropriate novel therapy. However, the current high cost of genetic testing and inability to demonstrate meaningful correlation between pathogenic mutations and phenotypical lipid disorders negatively impacts on the routine use of genetic testing (83).

CONCLUSION

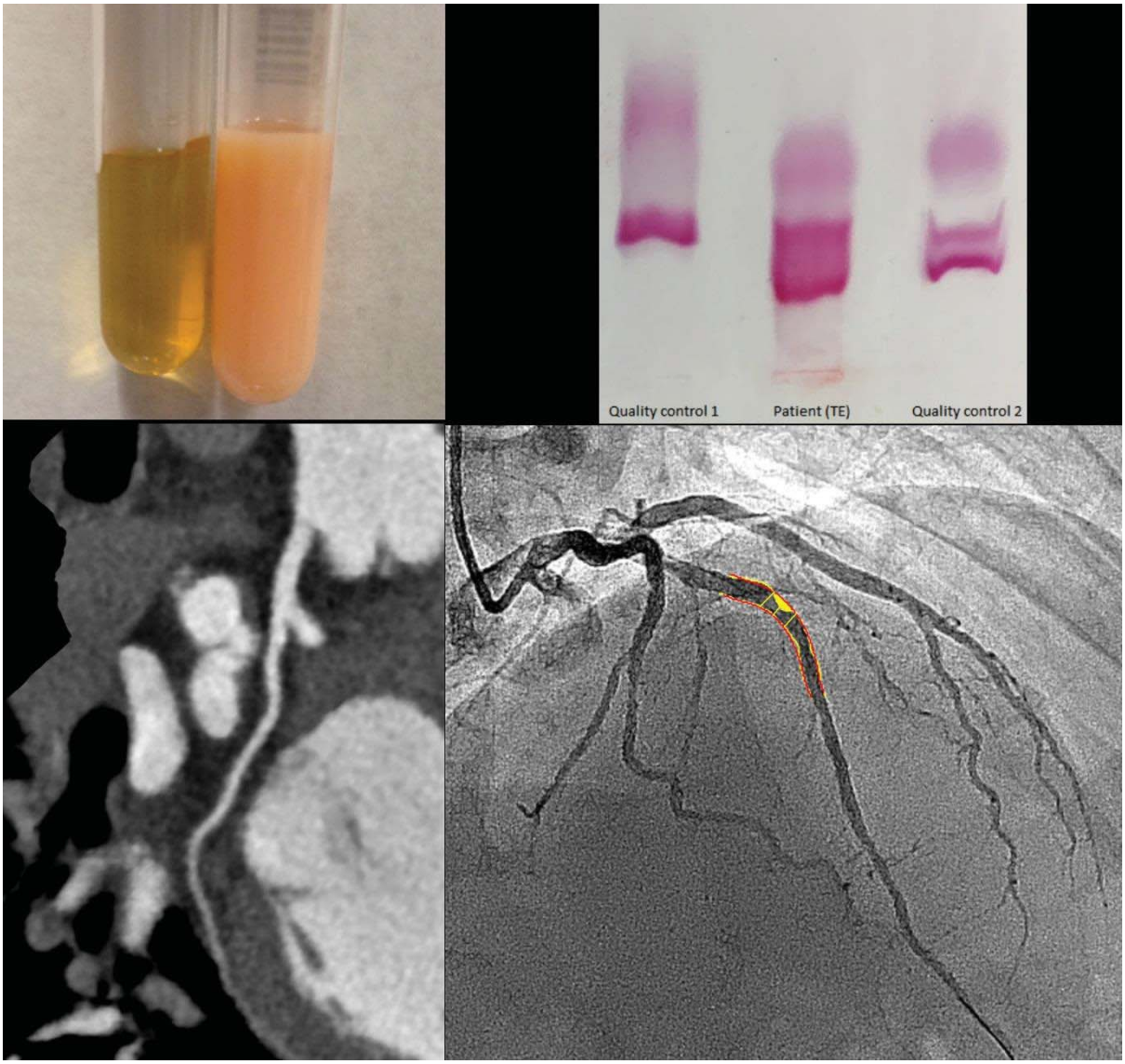
This review highlights the future treatment of atherosclerosis will be advancing to a multidisciplinary approach by cardiologists, lipidologists, biochemists and geneticist to establish a “personalised medicine” for an effective treatment. Among novel plasma lipid modulators, PCSK-9 inhibitors demonstrate strong efficacy in the treatment of marked hyperlipidaemia and, more importantly, a clear reduction in MACE and mortality outcomes, particularly in high risk population. The initial safety data of PCSK-9 mAb inhibitors is encouraging and its neutral impact on neurocognition is reassuring. Multiple challenges namely cost-effectiveness remain prior the institution of novel lipid-lowering agents in daily clinical practice, however in the near future, they are likely to play a major role in the management of complex refractory hyperlipidaemia with high risk of ASCVD.

Figure 1 Mechanisms of action of novel anti lipid agents.



Monoclonal antibodies to PCSK-9 act by binding to PCSK-9 and removing it from the circulation and preventing binding of PCSK-9 to the LDL receptor. SiRNAs act by degrading mRNA, thereby reducing PCSK-9 release to the circulation.AT04A is a PCSK-9-like antigen activating the immune system to produce PCSK-9 antibodies. HDL mimetics will improve efflux and reduce atherosclerosis process. Bempedoic acid (BA) reduces cholesterol synthesis through inhibition of adenosine triphosphate citrate lyase (ACL) upstream from 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA).

Figure 2 Biochemistry of atherosclerosis



The retention of lipoprotein components namely LDL-C, apo-B, Lipoprotein (a), and non-HDL-C (remnant cholesterols) in conjunction with endothelial dysfunction remain the robust hypothesis for the pathogenesis of atherosclerosis

Referrences

1. World Health Organisation: Cardiovascular diseases (CVDs) 2015.: WHO; September 2016 [Available from: http://www.who.int/mediacentre/factsheets/fs317/en/

2. Australian Bureau of Statistics, 2014, Causes of death Australia 2012, cat.no. 3303, last updated 30 March 2015, <http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/3303.0main+features100012012>.

3. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Primary care. 2013;40(1):195-211.

4. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. Heart. 2016;102(13):1003-8.

5. Marks D, Thorogood M, Neil HAW, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. Atherosclerosis. 2003;168(1):1-14.

6. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 Suppl 2):S1-45.

7. Grundy SM. Advances in treatment of dyslipidaemia. Nat Rev Cardiol. 2015.

8. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292(5519):1160-4.

9. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. The New England journal of medicine. 2004;350(15):1495-504.

10. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Eur Heart J. 2011;32(14):1769-818.

11. Buchwald H, Varco RL, Matts JP, Long JM, Fitch LL, Campbell GS, et al. Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). The New England journal of medicine. 1990;323(14):946-55.

12. Stefanutti C, Julius U, Watts GF, Harada-Shiba M, Cossu M, Schettler VJ, et al. Toward an international consensus-Integrating lipoprotein apheresis and new lipid-lowering drugs. Journal of clinical lipidology. 2017;11(4):858-71.e3.

13. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. European Heart Journal. 2016;37(39):2999-3058.

14. Rodriguez F, Harrington RA. Cholesterol, cardiovascular risk, statins, pcsk9 inhibitors, and the future of ldl-c lowering. Jama. 2016;316(19):1967-8.

15. Ference BA, Mahajan N. The role of early LDL lowering to prevent the onset of atherosclerotic disease. Current atherosclerosis reports. 2013;15(4):312.

16. Seidah NG. The proprotein convertases, 20 years later. Methods in molecular biology (Clifton, NJ). 2011;768:23-57.

17. Peterson AS, Fong LG, Young SG. PCSK9 function and physiology. Journal of lipid research. 2008;49(6):1152-6.

18. Park SW, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. The Journal of biological chemistry. 2004;279(48):50630-8.

19. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nature genetics. 2003;34(2):154-6.

20. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, et al. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. American journal of human genetics. 2006;79(3):514-23.

21. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama. 2007;298(3):299-308.

22. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis. 2016;253:281-344.

23. Aberg JA, Sponseller CA, Ward DJ, Kryzhanovski VA, Campbell SE, Thompson MA. Pitavastatin versus pravastatin in adults with HIV-1 infection and dyslipidaemia (INTREPID): 12 week and 52 week results of a phase 4, multicentre, randomised, double-blind, superiority trial. Lancet HIV. 2017;4(7):e284-e94.

24. Dong B, Wu M, Li H, Kraemer FB, Adeli K, Seidah NG, et al. Strong induction of PCSK9 gene expression through HNF1alpha and SREBP2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. Journal of lipid research. 2010;51(6):1486-95.

25. Zhang DW, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. The Journal of biological chemistry. 2007;282(25):18602-12.

26. Kastelein JJ, Nissen SE, Rader DJ, Hovingh GK, Wang MD, Shen T, et al. Safety and efficacy of LY3015014, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 (PCSK9): a randomized, placebo-controlled Phase 2 study. Eur Heart J. 2016;37(17):1360-9.

27. Ridker PM, Tardif JC, Amarenco P, Duggan W, Glynn RJ, Jukema JW, et al. Lipid-Reduction Variability and Antidrug-Antibody Formation with Bococizumab. The New England journal of medicine. 2017;376(16):1517-26.

28. Nicholls SJ, Puri R, Anderson T, Ballantyne CM, Cho L, Kastelein JJ, et al. Effect of Evolocumab on Progression of Coronary Disease in Statin-Treated Patients: The GLAGOV Randomized Clinical Trial. Jama. 2016;316(22):2373-84.

29. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. The New England journal of medicine. 2017;376(18):1713-22.

30. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. New England Journal of Medicine. 2018;379(22):2097-107.

31. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, et al. Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol. The New England journal of medicine. 2017;376(15):1430-40.

32. Landlinger C, Pouwer MG, Juno C, van der Hoorn JWA, Pieterman EJ, Jukema JW, et al. The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE*3Leiden.CETP mice. Eur Heart J. 2017;38(32):2499-507.

33. Urban D, Poss J, Bohm M, Laufs U. Targeting the proprotein convertase subtilisin/kexin type 9 for the treatment of dyslipidemia and atherosclerosis. Journal of the American College of Cardiology. 2013;62(16):1401-8.

34. Bilen O, Ballantyne CM. Bempedoic Acid (ETC-1002): an Investigational Inhibitor of ATP Citrate Lyase. Current Atherosclerosis Reports. 2016;18(10):61.

35. Ballantyne CM, Banach M, Mancini GBJ, Lepor NE, Hanselman JC, Zhao X, et al. Efficacy and safety of bempedoic acid added to ezetimibe in statin-intolerant patients with hypercholesterolemia: A randomized, placebo-controlled study. Atherosclerosis. 2018;277:195-203.

36. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nature medicine. 2004;10(12):1344-51.

37. C. WN, Jing P, D.M. RJ, F. WG. Nutraceuticals in the management of patients with statin-associated muscle symptoms, with a note on real-world experience. Clinical Cardiology. 2018;41(1):159-65.

38. Stein EA, Dufour R, Gagne C, Gaudet D, East C, Donovan JM, et al. Apolipoprotein B synthesis inhibition with mipomersen in heterozygous familial hypercholesterolemia: results of a randomized, double-blind, placebo-controlled trial to assess efficacy and safety as add-on therapy in patients with coronary artery disease. Circulation. 2012;126(19):2283-92.

39. Waldmann E, Vogt A, Crispin A, Altenhofer J, Riks I, Parhofer KG. Effect of mipomersen on LDL-cholesterol in patients with severe LDL-hypercholesterolaemia and atherosclerosis treated by lipoprotein apheresis (The MICA-Study). Atherosclerosis. 2017;259:20-5.

40. Duell PB, Santos RD, Kirwan BA, Witztum JL, Tsimikas S, Kastelein JJP. Long-term mipomersen treatment is associated with a reduction in cardiovascular events in patients with familial hypercholesterolemia. Journal of clinical lipidology. 2016;10(4):1011-21.

41. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama. 2001;285(19):2486-97.

42. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97(18):1837-47.

43. Tziomalos K. High-density lipoprotein: quantity or quality? Journal of thoracic disease. 2016;8(11):2975-7.

44. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. The New England journal of medicine. 2007;357(21):2109-22.

45. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. The New England journal of medicine. 2012;367(22):2089-99.

46. Nicholls SJ, Lincoff AM, Barter PJ, Brewer HB, Fox KA, Gibson CM, et al. Assessment of the clinical effects of cholesteryl ester transfer protein inhibition with evacetrapib in patients at high-risk for vascular outcomes: Rationale and design of the ACCELERATE trial. Am Heart J. 2015;170(6):1061-9.

47. Group THTRC. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. New England Journal of Medicine. 2017;377(13):1217-27.

48. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. Jama. 2003;290(17):2292-300.

49. Stephen J. Nicholls SEN. Result of the MILANO-PILOT Study 2016 [Available from: http://www.clinicaltrialresults.org/Slides/AHA2016/Nicholls_MILANO-PILOT.pdf.

50. Zhang M, He J, Jiang C, Zhang W, Yang Y, Wang Z, et al. Plaque-hyaluronidase-responsive high-density-lipoprotein-mimetic nanoparticles for multistage intimal-macrophage-targeted drug delivery and enhanced anti-atherosclerotic therapy. International Journal of Nanomedicine. 2017;12:533-58.

51. Duivenvoorden R, Tang J, Cormode DP, Mieszawska AJ, Izquierdo-Garcia D, Ozcan C, et al. A statin-loaded reconstituted high-density lipoprotein nanoparticle inhibits atherosclerotic plaque inflammation. Nature communications. 2014;5:3065.

52. Jacobson TA. Lipoprotein(a), cardiovascular disease, and contemporary management. Mayo Clin Proc. 2013;88(11):1294-311.

53. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis. 2016;253(Supplement C):281-344.

54. Schettler VJJ, Ringel J, Jacob S, Julius U, Klingel R, Heigl F, et al. Current insights into the German lipoprotein apheresis standard: PCSK9-inhibitors, lipoprotein apheresis or both? Atherosclerosis Supplements. 2017;30:44-9.

55. Seed M, O'Connor B, Perombelon N, O'Donnell M, Reaveley D, Knight BL. The effect of nicotinic acid and acipimox on lipoprotein(a) concentration and turnover. Atherosclerosis. 1993;101(1):61-8.

56. Makimura H, Stanley TL, Suresh C, De Sousa-Coelho AL, Frontera WR, Syu S, et al. Metabolic Effects of Long-Term Reduction in Free Fatty Acids With Acipimox in Obesity: A Randomized Trial. The Journal of clinical endocrinology and metabolism. 2016;101(3):1123-33.

57. Halbirk M, Nørrelund H, Møller N, Schmitz O, Gøtzsche L, Nielsen R, et al. Suppression of circulating free fatty acids with acipimox in chronic heart failure patients changes whole body metabolism but does not affect cardiac function. American Journal of Physiology - Heart and Circulatory Physiology. 2010;299(4):H1220-H5.

58. Group TH-TC. Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. New England Journal of Medicine. 2014;371(3):203-12.

59. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. Lancet (London, England). 2016;388(10057):2239-53.

60. Rosenson RS, Davidson MH, Hirsh BJ, Kathiresan S, Gaudet D. Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease. Journal of the American College of Cardiology. 2014;64(23):2525-40.

61. Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA, et al. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. Lancet (London, England). 2013;381(9860):40-6.

62. Araki E, Yamashita S, Arai H, Yokote K, Satoh J, Inoguchi T, et al. Effects of Pemafibrate, a Novel Selective PPARalpha Modulator, on Lipid and Glucose Metabolism in Patients With Type 2 Diabetes and Hypertriglyceridemia: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial. Diabetes care. 2018;41(3):538-46.

63. Bhatt DL, Steg PG, Brinton EA, Jacobson TA, Miller M, Tardif J-C, et al. Rationale and design of REDUCE-IT: Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial. Clinical cardiology. 2017;40(3):138-48.

64. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. New England Journal of Medicine.0(0):null.

65. Nicholls SJ, Lincoff AM, Bash D, Ballantyne CM, Barter PJ, Davidson MH, et al. Assessment of omega-3 carboxylic acids in statin-treated patients with high levels of triglycerides and low levels of high-density lipoprotein cholesterol: Rationale and design of the STRENGTH trial. Clin Cardiol. 2018;41(10):1281-8.

66. Sharma K, Baliga RR. Genetics of Dyslipidemia and Ischemic Heart Disease. Current Cardiology Reports. 2017;19(5):46.

67. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, et al. Antisense Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia. New England Journal of Medicine. 2015;373(5):438-47.

68. Hegele RA, Berberich AJ, Ban MR, Wang J, Digenio A, Alexander VJ, et al. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol. 2018;12(4):920-7.e4.

69. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, et al. Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. New England Journal of Medicine. 2017;377(3):211-21.

70. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 Inhibition in Homozygous Familial Hypercholesterolemia. New England Journal of Medicine. 2017;377(3):296-7.

71. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2015;385(9965):331-40.

72. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2015;385(9965):341-50.

73. Kastelein JJ, Robinson JG, Farnier M, Krempf M, Langslet G, Lorenzato C, et al. Efficacy and safety of alirocumab in patients with heterozygous familial hypercholesterolemia not adequately controlled with current lipid-lowering therapy: design and rationale of the ODYSSEY FH studies. Cardiovasc Drugs Ther. 2014;28(3):281-9.

74. Waldmann E, Parhofer KG. Lipoprotein apheresis to treat elevated lipoprotein (a). Journal of Lipid Research. 2016;57(10):1751-7.

75. Sahebkar A, Watts GF. New therapies targeting apoB metabolism for high-risk patients with inherited dyslipidaemias: what can the clinician expect? Cardiovasc Drugs Ther. 2013;27(6):559-67.

76. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, et al. Antisense Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia. The New England journal of medicine. 2015;373(5):438-47.

77. Stroes E, Colquhoun D, Sullivan D, Civeira F, Rosenson RS, Watts GF, et al. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. Journal of the American College of Cardiology. 2014;63(23):2541-8.

78. Moriarty PM, Thompson PD, Cannon CP, Guyton JR, Bergeron J, Zieve FJ, et al. Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: The ODYSSEY ALTERNATIVE randomized trial. Journal of clinical lipidology. 2015;9(6):758-69.

79. Moriarty PM, Parhofer KG, Babirak SP, Cornier MA, Duell PB, Hohenstein B, et al. Alirocumab in patients with heterozygous familial hypercholesterolaemia undergoing lipoprotein apheresis: the ODYSSEY ESCAPE trial. Eur Heart J. 2016;37(48):3588-95.

80. Vogt A, Parhofer KG. The potential of mipomersen, an ApoB synthesis inhibitor, to reduce necessity for LDL-apheresis in patients with heterozygous familial hypercholesterolemia and coronary artery disease. Expert opinion on pharmacotherapy. 2013;14(6):691-7.

81. Korman MJ, Retterstol K, Kristiansen IS, Wisloff T. Are PCSK9 Inhibitors Cost Effective? PharmacoEconomics. 2018;36(9):1031-41.

82. Giugliano RP, Mach F, Zavitz K, Kurtz C, Im K, Kanevsky E, et al. Cognitive Function in a Randomized Trial of Evolocumab. New England Journal of Medicine. 2017;377(7):633-43.

83. Page MM, Stefanutti C, Sniderman A, Watts GF. Recent advances in the understanding and care of familial hypercholesterolaemia: significance of the biology and therapeutic regulation of proprotein convertase subtilisin/kexin type 9. Clinical science (London, England : 1979). 2015;129(1):63-79.

Chapter 2 B

The Role of Low Density Lipoprotein Apheresis in the Management of Heterozygous Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis

The Role of Low Density Lipoprotein Apheresis in the Management of Heterozygous Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis

Sam Mirzaee MD MPH [a], Paul M Thein MBBS [a], Sarah Zaman MBBS, PhD[a], James D Cameron MBBS MD BE (Elec) MEngSc[a]. Arthur Nasis MBBS, MD, PhD [a]

[a] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia

Corresponding Author:

Dr Sam Mirzaee

Monash Cardiovascular Research Centre, MonashHEART, Monash Health

246 Clayton Road, Clayton, Vic 3150 Australia

Telephone: +61 9594 6666

Email: Sam.Mirzaee@Monashealth.org

ABBREVIATIONS

ACS	Acute Coronary Syndrome
ASCVD	Atherosclerotic Cardiovascular Disease
BMI	Body Mass Index
CI	Confidence Interval
CAD	Coronary Artery Disease
HoFH	Homozygous familial hypercholesterolemia
HeFH	Heterozygous familial hypercholesterolemia
HDL	High Density Lipoprotein
HMG CO-A	Hydroxy-methylglutaryl-coenzyme A
IVUS	Intravascular Ultrasound
LDL-C	Low Density Lipoprotein Cholesterol
LDL-A	Low Density Lipoprotein Apheresis
Lp (a)	Lipoprotein (a)
MACCE	Major Adverse Cardiac and Cerebrovascular Events
MDCT	Multi-detector Computed Tomography
MI	Myocardial Infarction
MLD	Minimum Luminal Diameter
MOD	Minimal Obstruction Diameter
PDS	Percent Diameter Stenosis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PCSK9	Proprotein convertase subtilisin/kexin type 9
RVD	Reference Vessel Diameter
RCT	Randomised Clinical Trial
SD	Standard Deviation

ABSTRACT

Background: Familial hypercholesterolemia (FH), with its associated high risk of premature cardiovascular events, is becoming increasingly recognized and can be reliably treated in earlier stages of life. There is ongoing debate whether aggressive low density lipoprotein cholesterol (LDL-C) lowering by additional LDL-C apheresis (LDL-A) leads to improved cardiovascular outcomes.

Methods: Electronic databases were searched for clinical trials that included treatment with LDL-A in combination with medical therapy in patients with heterozygous FH (HeFH) associated coronary artery disease. The primary end point was a change in the severity of coronary artery disease measured by minimal luminal diameter (MLD) via quantitative coronary angiography (QCA) or intravascular ultrasound (IVUS). Secondary endpoints included LDL-C level, cardiac symptoms, major adverse cerebrovascular and cardiovascular events (MACCE), myocardial infarction and death.

Results: In total, 6 studies met inclusion criteria with 38.7% (148/382) of included patients having LDL-apheresis during a median follow-up of 24 months (interquartile range 18-25). MLD did not significantly change in patients who underwent LDL-A (Mean difference (MD) 0.15, 95% CI -0.04, 0.35, I^2=89%, *P=0.11*). However, LDL-C level was significantly reduced in the LDL-A population (MD -1.05 mmol/L, CI -2.00, -0.10, I^2=84%, *P=0.03*), with a trend towards reduction of major adverse cardiovascular and cerebrovascular events (risk difference -0.17, CI -0.34, 0.01, I^2= 47%, *P=0.06*).

Conclusions: This review suggests that LDL-A leads to a significant lowering of LDL-C level with a trend to a lower rate of subsequent cardiovascular events in patients with HeFH and known coronary artery disease. This may be explained by the potential inhibitory impact of LDL-A on plaque progression.

Keywords: Lipid apheresis, Coronary plaque progression, Familial hypercholesterolemia, Quantitative coronary angiography

INTRODUCTION

Heterozygous Familial hypercholesterolemia (HeFH) is a common genetic disorder of lipid metabolism characterised by the accumulation of plasma LDL-C as a long-term consequence of mutations in the LDL-C receptor, Apo lipoprotein-B or Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) ((1)). The association of FH with CAD has been well established since 1939 when Carl Muller first described a case of angina pectoris in a patient with FH (2).Yet this condition continues to contribute to premature atherosclerotic cardiovascular disease (ASCVD) in young adults, with reported high mortality rates (3). Although premature ASCVD in patients with FH mainly manifests in adulthood, it has been described as early as the first decade of life (4). Therefore an effective and timely LDL-C lowering strategy is critical in the management of all patients with FH.

Recent data shows high intensity statin monotherapy is inadequate for treatment of patients with HeFH to achieve recommended LDL-C levels (5). LDL-A as a technique that involves the extracorporeal elimination of serum LDL-C that has been suggested as a treatment option for selected HeFH patients in whom LDL-C levels remain substantially elevated despite optimisation of medical management (6, 7). The efficacy and safety of additional LDL-A in lowering LDL-C levels has been demonstrated in several landmark studies (8). In addition to the beneficial effects on lowering LDL-C, supplemental LDL-A promotes pleiotrophic effects including enhancement of endothelial function via nitric oxide production and immune-regulation which in turn can improve ischemic symptoms, functional capacity and myocardial perfusion (9, 10).

Despite mounting evidence supporting lipid apheresis as a therapeutic technique that improves lipid profile and provides cardio-physiological benefits, the actual impact of LDL-A on the progression of coronary atherosclerosis remains unclear. Additionally, a recent trial that investigated the efficacy of PCSK9 monoclonal antibody inhibition compared to LDL-A in patients with heterozygous FH (HeFH) demonstrated that over 60% of patients did not require further apheresis, suggesting a substitute therapy for selected FH patients and therefore the cost-effectiveness of LDL-A needs to be

reassessed (11). We performed a systematic review and meta-analysis to study the effect of LDL-A combined with medical therapy on minimal luminal diameter (MLD) assessed by QCA or intravascular ultrasound (IVUS), as compared with medical therapy alone in patients with FH.

METHODS

Data sources and search strategy

Comprehensive searches of EMBASE, MEDLINE and The Cochrane Library along with trial registries and abstracts from major scientific meetings were performed to identify eligible publications. The review was conducted consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (12). Our search was not restricted to studies published in English language only. The review protocol was prospectively registered under PROSPERO International prospective register of systematic reviews (PROSPERO 2017d: CRD42017072231). **Figure 1** illustrates The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart. An example search strategy for MEDLINE is provided in **Supplemental Table S1.**

Study selection

Inclusion criteria for studies were as follows: 1) reporting of coronary artery disease via QCA or IVUS in adult patients with HeFH, 2) comparison of changes in minimal luminal diameter (∆MLD) between LDL-A and medical therapy alone patients, 3) follow up duration of ≥6 months, 4) prospectively controlled trials, 5) fully published status. Studies were excluded if they did not report CAD, did not have a control group, or if atherosclerosis was assessed with the methodologies described above. The details of inclusion and exclusion criteria for each study has been summarised in **Supplemental Table S2.** In the event of multiple publications reporting results on the same cohort of participants, we included the most recent papers with largest sample size and follow up period. Study specific definitions of coronary artery disease progression or regression were used in this analysis that encompassed changes in MLD from baseline to follow up (∆MLD). MLD was calculated as the

smallest lumen diameter in the segment of interest while diameter stenosis (DS) was calculated as a percentage of the difference of reference vessel diameter (RVD) and MLD divided by RVD, as previously described (13) **(Figure 2)**. Specific details for the assessment performed in each individual study are available in **Supplemental Table S3.**

Data items and collection process

Prior to the literature search, data items were specified. Two authors (SM and PMT) independently conducted the search and data extraction for baseline patient characteristics and clinical outcomes. Extracted data was verified by the senior author and any discrepancies resolved by consensus agreement. Within each individual trial, the risk of bias was assessed according to the Cochrane Collaboration Assessment tool **(Supplemental Figure 1)**.

Study endpoints

The primary endpoint of the study was defined as the change in MLD measured from baseline to follow-up, comparing groups of subjects on medications alone versus the combination of medical therapy and LDL-A. Secondary endpoints included changes in both LDL-C concentrations and High Density Lipoprotein Cholesterol (HDL-C), cardiovascular composite outcome [angina symptoms, MACCE (major adverse cardiac and cerebrovascular events), myocardial infarction, coronary revascularisation, and cardiac death] and the incidence of potential complications including overall mortality, systemic thromboembolism and clinically significant hypotension. Full details of individual clinical endpoints and definitions are presented in **Supplemental Table S4.**

Statistical analysis

Meta-analysis was performed using Review Manager (RevMan), version 5.3 (Cochrane Collaboration, Oxford, UK). Outcomes were analysed using a random effects model and summary estimates reported as the weighted mean difference (MD) with 95% confidence intervals (CI) for the primary endpoint of change in MLD. For the secondary outcome of cardiovascular events, pooled

odds ratios (OR) with 95% confidence intervals (CI) were used. Continuous variables are reported as the mean and standard deviation (SD), with non-parametric data converted to mean (SD) as previously described (14). For all analyses, a two-sided p-value of <0.05 was regarded as significant. The quality of outcome was weighed via Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system with publication bias assessed visually via funnel plots **(Supplement figure 2)**. Heterogeneity was evaluated for all endpoints with the I^2 statistic. Heterogeneity was considered as low, moderate or high based on an I^2 values of 25%, 50% and 75% respectively (15).

RESULTS

Search results

A total of 15,707 initial citations were returned from the electronic searches. Following screening, 19 studies were identified for potential inclusion and further assessment. Of these, 13 studies were excluded as either having no coronary vessel assessment (n=2), no medical control group or specific primary outcome (n=6), measurement tool ineligible for review (n=3) and duplications (n=2). Full details of trial selection are presented in **Figure 1**.

Six studies met the predefined criteria and were included in the final analysis (10, 16-20) . Eligible trials included combined data from the The Low-Density Lipoprotein Apheresis Coronary Atherosclerosis Prospective Study (L-CAPS), Familial Hypercholesterolemia Regression Study (FHRS), LDL Apheresis Regression Study (LAARS), Low-Density Lipoprotein Apheresis Coronary Morphology and Reserve Trial (LACMART).

Overall, 338 subjects were included in the analysis with 148 subjects receiving combined LDL-A and drug therapy and 195 receiving drug therapy alone (10, 16-20). The median follow-up of 24 months (interquartile range 18-25) was observed among the studies. Full details of the patient's characteristics and the clinical demographics for each study are presented in **Table 1 and Table 2**, respectively.

Changes in minimal luminal diameter

Five of the six studies reported the primary endpoint of change in MLD between baseline and follow-up (8, 13, 15-17). Treatment with a combination of LDL-A and medical therapy was not associated with significant change in MLD, when compared with pharmacotherapy alone (MD 0.15 mm, 95% CI 0.02 to 0.39, I^2=89%, p=0.11) with high heterogeneity observed between studies **(Figure 3)**. Sensitivity analyses were not performed due to high heterogeneity and potential underpowered sample size.

Secondary endpoints

All studies reported data on LDL-C concentrations, with co-administration of LDL-A and pharmacotherapy being associated with a significantly lower LDL-C concentration when compared with medication therapy alone (MD –1.05 mmol/L, CI -2.0 to –0.10, I^2 84%, p = 0.03) **(Figure 4)**. However, we were unable to assess this reduction in relationship with other lipid profile parameters, particularly HDL-C level, due to variation in reported data. Finally, we observed reduction in risk difference (RD) in favour of LDL-A treatment, with a trend to reduced risk for major adverse cardiac and cerebrovascular events (MACCE) including angina symptoms, acute myocardial infarction (AMI), revascularisation by coronary artery bypass grafting or percutaneous intervention, stroke and transient ischemic attack (RD = -0.17, CI - 0.34 to 0.01, I^2=47%, p = 0.06) **(Figure 5)**. Meta-regression to assess the correlation between LDL reduction and MLD changes was not performed due to substantial heterogeneity among studies.

DISCUSSION

To our knowledge, this is the first meta-analysis investigating whether supplementary LDL-A in addition to pharmacotherapy is associated with changes in the course of coronary artery disease. Our main finding is that additional LDL-A, in addition to pharmacotherapy, did not result in a statistically significant difference of luminal stenosis measured by MLD via QCA or IVUS when compared with medical therapy alone. However, three studies showed regression of luminal stenosis in the LDL-A group. The extent of MLD change appeared to be associated with baseline LDL, with greater

increases in MLD found in studies reporting lower baseline LDL-C. Additionally, we found that supplementary LDL-A in combination with drug therapy was associated with significant reduction in circulating LDL-C levels, and subsequent cardiovascular outcomes. Our results suggest that improvements in clinical outcomes with LDL-A therapy may be driven by halting coronary artery disease progression when compared with pharmacotherapy alone.

LDL-A, in addition to diet and maximum tolerated pharmacotherapy, is an established treatment for patients with homozygous FH (HoFH), severe HeFH or refractory hypercholesterolemia (6, 21-23). Generally, LDL-A is a costly but effective and safe means of reducing LDL-C in select patients, however this may be associated with adverse events such as hypotension, nausea and vomiting (24). The large body of epidemiological and genetic evidence implies the causality of LDL-C in atherogenesis and support benefits from LDL-C reduction (25). Further cardiovascular events depend upon pre-treatment LDL-C level and the extent of LDL-C lowering (26). Support for this concept is also provided in our analysis, as we found that additional LDL-C lowering via apheresis improved cardiac outcomes.

In our review, we also observed that the average age of developing clinically significant CAD in patients with phenotypically diagnosed FH is one to two decades earlier than the general population, consistent with existing data in the literature (27). Hence, in patients with hereditary lipid disorders, LDL-C lowering should be considered in earlier stages of life due to the high risk nature of this condition. As the advancement of CAD and further cardiovascular events in patients with FH is not only determined by LDL-C level in plasma, perhaps there are also pleiotrophic effects in using long-term LDL-A in this high risk population which could explain our results (28, 29). These proposed mechanisms resulting in clinical benefit of LDL-A in HeFH include improvement of myocardial perfusion, coronary flow reserve, microcirculation, endothelial function and overall cardiovascular homeostasis (29, 30). Further studies are warranted to assess alteration in quantity and composition of plaque atheroma using high technology tools such as IVUS and optical coherence tomography (OCT).

LDL-A is also an accepted therapy for patients with refractory hypercholesterolemia and those HeFH patients who have progressive coronary disease and elevated lipoprotein(a) [Lp(a)] (31). A recent prospective clinical trial demonstrated positive cardiovascular benefits of concurrent selective Lp(a) apheresis in addition to pharmacotherapy (32). As the majority of studies showing a cardiovascular benefit of LDL-A are observational and were performed in the 1990s, there is a need for further prospective clinical trials using contemporary medical therapy (33). Also, due to the emerigence of novel LDL-C lowering agents, particularly PCSK9 monoclonal antibodies, the cost effectiveness and the value of lipid apheresis in HeFH needs further evaluation. ODYSSEY ESCAPE studied the effect of alirocumab on the required frequency of lipid apheresis treatments in HeFH, suggesting the pathway for initiation and frequency of apheresis can be modified by using novel lipid lowering agents but lipid apheresis would remain warranted in selected complex patients with HeFH (11). Future studies and formal health economic assessment is also imperative to explore the relative and adjunctive effects of novel lipid lowering agents and lipid apheresis, particularly selective Lp(a) apheresis in subsets with elevated Lp(a) and severe FH (34).

Study limitations

These results must be interpreted in view of their limitations. Firstly, using QCA to assess MLD as a method for quantification of CAD has low sensitivity (35). Secondly, the relatively small sample size, the heterogeneity of subgroup populations [with or without elevated Lp(a)], and difference in type and dose of lipid lowering medications used in each trial may have impacted the outcomes. Additionally, QCA was not completed in all coronary vessels, limiting accurate quantification of coronary disease regression or progression throughout the entire coronary tree. Also, there was no consistency amongst the studies in terms of the time interval between pre and post-treatment QCA assessment and LDL-C measurements and this may have impacted on the degree of luminal stenosis and LDL-C alteration. Kutsumi et al did not specify the study population as HeFH, yet the basic characteristics of subjects appear to be HeFH. Finally, LDL-A may have been initiated after the diagnosis of CAD, therefore the true effect of this treatment in the prevention of CAD remains ambiguous.

CONCLUSIONS

Combined LDL-A and pharmacotherapy in patients with phenotypical HeFH is associated with significant reduction in LDL-C with a trend to reduction in subsequent cardiovascular events. Further prospective randomised controlled trials are required to ascertain cardiovascular benefits and cost effectiveness of combined lipid apheresis in addition to standard pharmacological therapy in this patient population.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST AND FUNDING

SM has received consultancy fees from both Amgen and MSD. The other authors do not have any conflict of interest.

References:

1. Varghese M. Familial hypercholesterolemia: A review. Annals of Pediatric Cardiology. 2014;7(2):107-17.

2. Muller C. Angina pectoris in hereditary xanthomatosis. JAMA. 1939;64(4):675-700.

3. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease Consensus Statement of the European Atherosclerosis Society. European Heart Journal. 2013;34(45):3478-90.

4. Varghese MJ. Familial hypercholesterolemia: A review. Annals of Pediatric Cardiology. 2014;7(2):107-17.

5. Faggiano P, Pirillo A, Griffo R, Ambrosetti M, Pedretti R, Scorcu G, et al. Prevalence and management of familial hypercholesterolemia in patients with coronary artery disease: The heredity survey. International journal of cardiology. 2018;252:193-8.

6. Schwartz J, Padmanabhan A, Aqui N, Balogun RA, Connelly-Smith L, Delaney M, et al. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice–Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Seventh Special Issue. Journal of Clinical Apheresis. 2016;31(3):149-338.

7. Szczepiorkowski ZM, Shaz BH, Bandarenko N, Winters JL. The new approach to assignment of ASFA categories--introduction to the fourth special issue: clinical applications of therapeutic apheresis. J Clin Apher. 2007;22(3):96-105.

8. Wang A, Richhariya A, Gandra SR, Calimlim B, Kim L, Quek RGW, et al. Systematic Review of Low-Density Lipoprotein Cholesterol Apheresis for the Treatment of Familial Hypercholesterolemia. Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease. 2016;5(7):e003294.

9. Sinzinger H, Steiner S, Derfler K. Pleiotropic effects of regular lipoprotein-apheresis. Atherosclerosis Supplements. 2017;30(Supplement C):122-7.

10. Matsuzaki M, Hiramori K, Imaizumi T, Kitabatake A, Hishida H, Nomura M, et al. Intravascular ultrasound evaluation of coronary plaque regression by low density lipoprotein-apheresis in familial hypercholesterolemia: the Low Density Lipoprotein-Apheresis Coronary Morphology and Reserve Trial (LACMART). Journal of the American College of Cardiology. 2002;40(2):220-7.

11. Moriarty PM, Parhofer KG, Babirak SP, Cornier MA, Duell PB, Hohenstein B, et al. Alirocumab in patients with heterozygous familial hypercholesterolaemia undergoing lipoprotein apheresis: the ODYSSEY ESCAPE trial. Eur Heart J. 2016;37(48):3588-95.

12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009;6(7):e1000097.

13. Arbab-Zadeh A, Hoe J. Quantification of Coronary Arterial Stenoses by Multidetector CT Angiography in Comparison With Conventional Angiography: Methods, Caveats, and Implications. JACC: Cardiovascular Imaging. 2011;4(2):191-202.

14. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC medical research methodology. 2005;5:13.

15. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed). 2003;327(7414):557-60.

16. Thompson GR, Maher VMG, Kitano Y, Neuwirth C, Maher VMG, Davies G, et al. Familial Hypercholesterolaemia Regression Study: a randomised trial of low-density-lipoprotein apheresis. The Lancet.345(8953):811-6.

17. Daida H, Lee YJ, Yokoi H, Kanoh T, Ishiwata S, Kato K, et al. Prevention of restenosis after percutaneous transluminal coronary angioplasty by reducing lipoprotein (a) levels with low-density lipoprotein apheresis. Low-Density Lipoprotein Apheresis Angioplasty Restenosis Trial (L-ART) Group. The American journal of cardiology. 1994;73(15):1037-40.

18. Kutsumi Y, Tada H, Iwamuro A, Kabuto H. Is low density lipoprotein apheresis effective for coronary artery disease? ASAIO journal (American Society for Artificial Internal Organs : 1992). 1998;44(5):M666-9.

19. Kroon AA, Aengevaeren WR, van der Werf T, Uijen GJ, Reiber JH, Bruschke AV, et al. LDL-Apheresis Atherosclerosis Regression Study (LAARS). Effect of aggressive versus conventional lipid lowering treatment on coronary atherosclerosis. Circulation. 1996;93(10):1826-35.

20. Nishimura S, Sekiguchi M, Kano T, Ishiwata S, Nagasaki F, Nishide T, et al. Effects of intensive lipid lowering by low-density lipoprotein apheresis on regression of coronary atherosclerosis in patients with familial hypercholesterolemia: Japan Low-density Lipoprotein Apheresis Coronary Atherosclerosis Prospective Study (L-CAPS). Atherosclerosis. 1999;144(2):409-17.

21. Thompson GR. Recommendations for the use of LDL apheresis. Atherosclerosis. 2008;198(2):247-55.

22. Harada-Shiba M, Arai H, Oikawa S, Ohta T, Okada T, Okamura T, et al. Guidelines for the management of familial hypercholesterolemia. Journal of atherosclerosis and thrombosis. 2012;19(12):1043-60.

23. Schwartz J, Padmanabhan A, Aqui N, Balogun RA, Connelly-Smith L, Delaney M, et al. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice-Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Seventh Special Issue. Journal of clinical apheresis. 2016;31(3):149-62.

24. Wang A, Richhariya A, Gandra SR, Calimlim B, Kim L, Quek RG, et al. Systematic Review of Low-Density Lipoprotein Cholesterol Apheresis for the Treatment of Familial Hypercholesterolemia. Journal of the American Heart Association. 2016;5(7).

25. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. European Heart Journal. 2017;38(32):2459-72.

26. Soran H, Dent R, Durrington P. Evidence-based goals in LDL-C reduction. Clinical Research in Cardiology. 2017;106(4):237-48.

27. Setia N, Verma IC, Khan B, Arora A. Premature Coronary Artery Disease and Familial Hypercholesterolemia: Need for Early Diagnosis and Cascade Screening in the Indian Population. Cardiology Research and Practice. 2012;2012:658526.

28. Ramunni A, Burzo M, Verno L, Brescia P. Pleiotropic effects of LDL apheresis. Atherosclerosis Supplements. 2009;10(5):53-5.

29. Sampietro T, Sbrana F, Pasanisi EM, Bigazzi F, Petersen C, Coceani M, et al. LDL apheresis improves coronary flow reserve on the left anterior descending artery in patients with familial hypercholesterolemia and chronic ischemic heart disease. Atherosclerosis Supplements. 2017;30:135-40.

30. Sampietro T, Tuoni M, Ferdeghini M, Ciardi A, Marraccini P, Prontera C, et al. Plasma cholesterol regulates soluble cell adhesion molecule expression in familial hypercholesterolemia. Circulation. 1997;96(5):1381-5.

31. Moriarty P, Gibson C. Low-density lipoprotein apheresis in the treatment of atherosclerosis and other potential uses2001. 156-62 p.

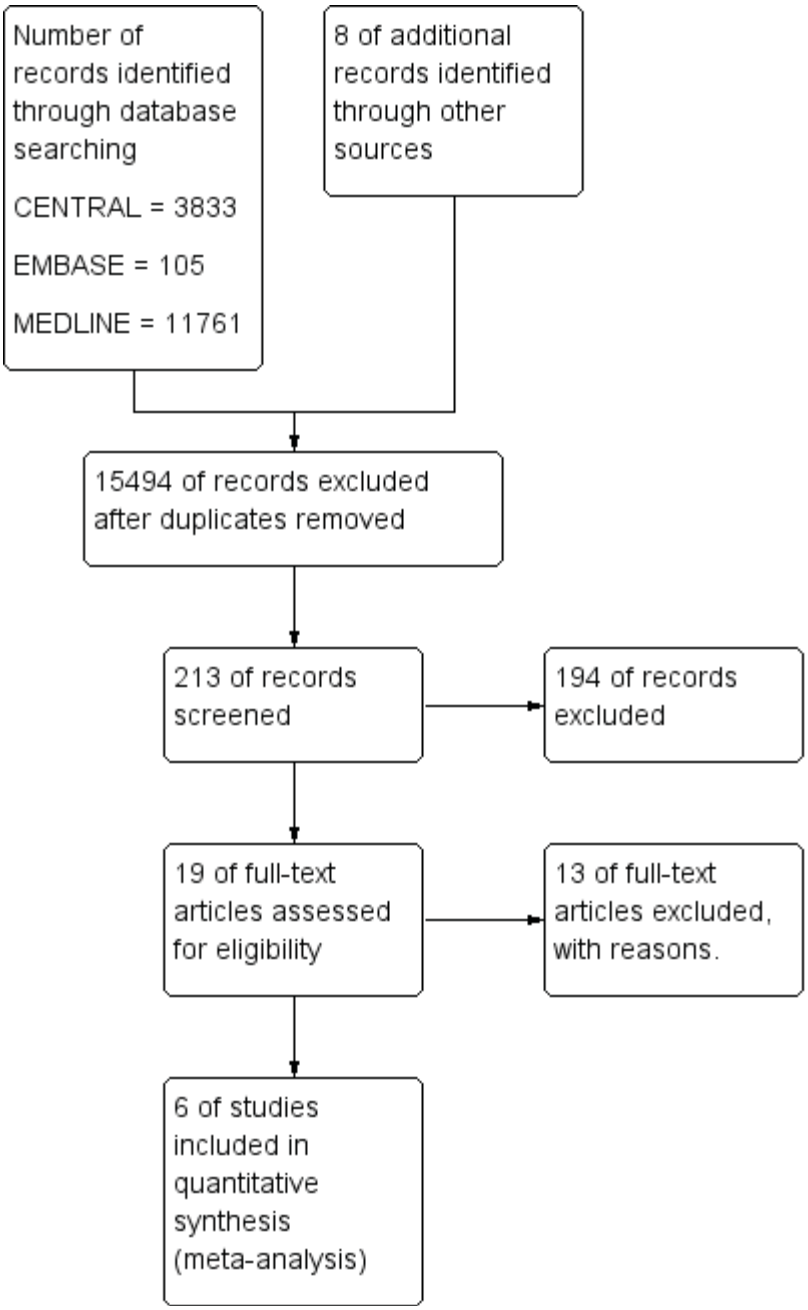
32. Safarova MS, Ezhov MV, Afanasieva OI, Matchin YG, Atanesyan RV, Adamova IY, et al. Effect of specific lipoprotein(a) apheresis on coronary atherosclerosis regression assessed by quantitative coronary angiography. Atherosclerosis Supplements. 2013;14(1):93-9.

33. Tatami R, Inoue N, Itoh H, Kishino B, Koga N, Nakashima Y, et al. Regression of coronary atherosclerosis by combined LDL-apheresis and lipid-lowering drug therapy in patients with familial hypercholesterolemia: a multicenter study. The LARS Investigators. Atherosclerosis. 1992;95(1):1-13.

34. Watts GF, Stefanutti C. ODYSSEY ESCAPE: is PCSK9 inhibition the Trojan Horse for the use of lipoprotein apheresis in familial hypercholesterolaemia? European Heart Journal. 2016;37(48):3596-9.

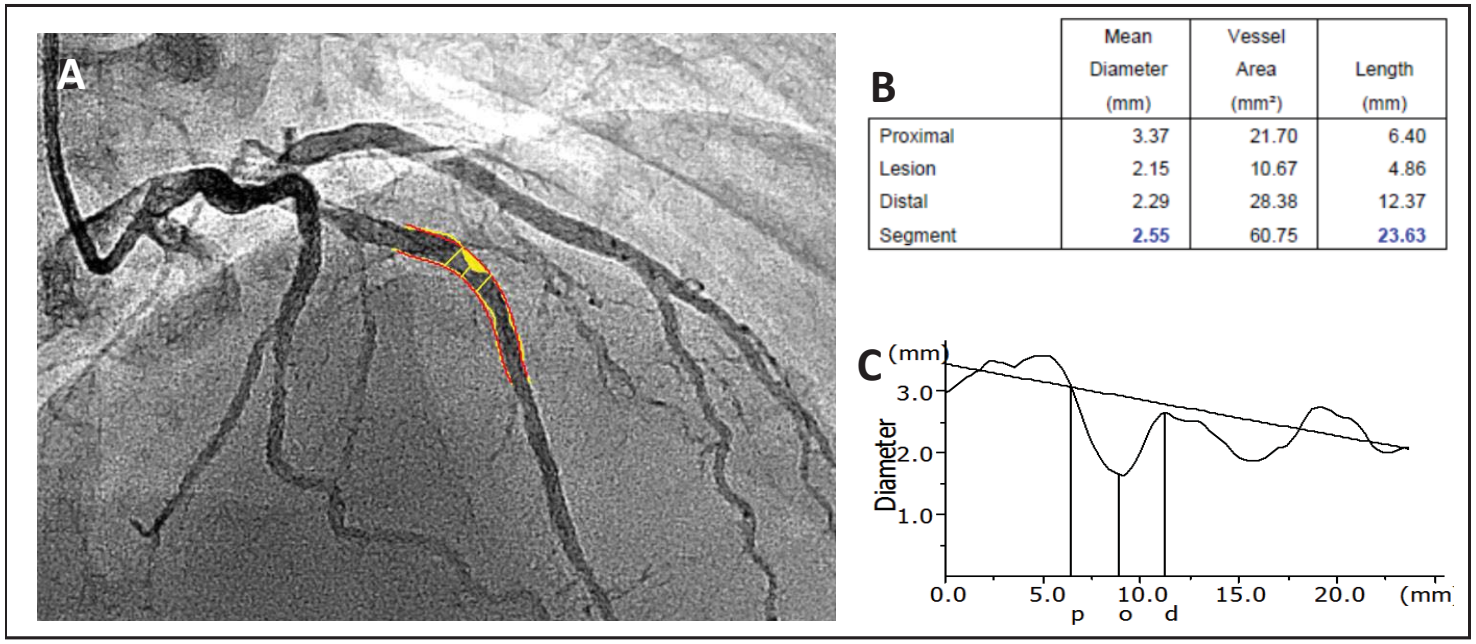
35. Meijboom WB, Van Mieghem CAG, van Pelt N, Weustink A, Pugliese F, Mollet NR, et al. Comprehensive Assessment of Coronary Artery Stenoses: Computed Tomography Coronary Angiography Versus Conventional Coronary Angiography and Correlation With Fractional Flow Reserve in Patients With Stable Angina. Journal of the American College of Cardiology. 2008;52(8):636-43.

Figure 1. Study Flow Chart



Flow diagram illustrating the study selection process for the systematic review and meta-analysis

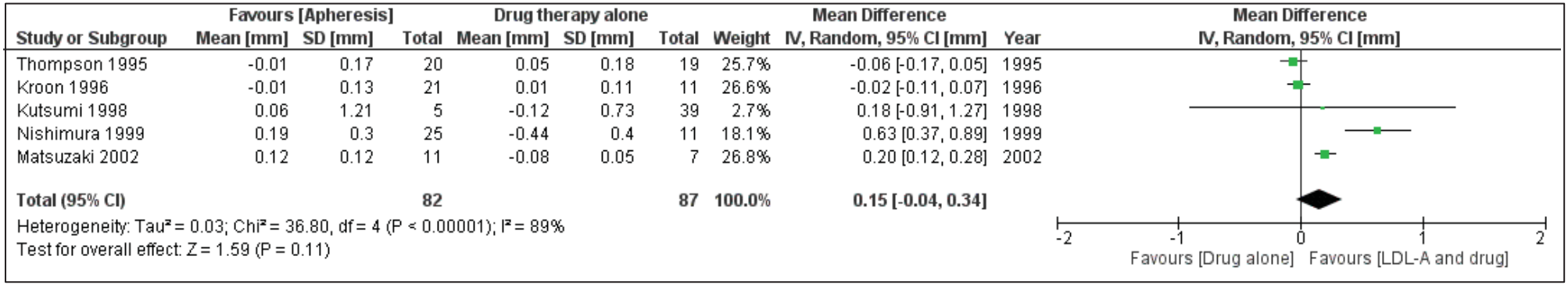
Figure 2. Quantitative coronary angiogram



(A) 2D QCA is a semi-automated tracing vessel edge (B) Parameters obtained from semi-automated software (C) Minimum luminal diameter in association with proximal (p) and distal (d) segment

Figure 3. Changes in the course of coronary artery disease assessed by minimal luminal diameter

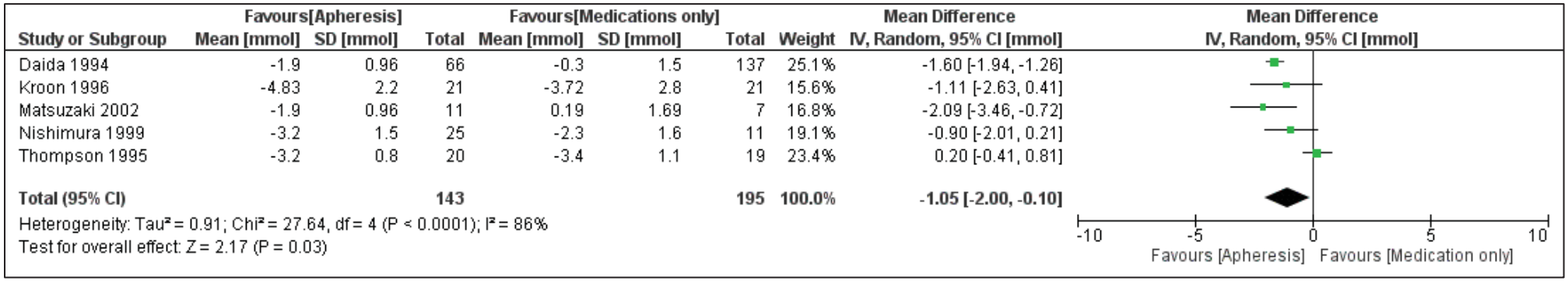
Forest plot displays summary Mean Difference (MD) and 95% confidence intervals (CI).



LDL-A; low density lipoprotein apheresis

Figure 4. Effectiveness of additional low density lipoprotein apheresis on LDL

Forest plot displays summary mean difference (MD) and 95% confidence intervals (CI).



LDL; low density lipoprotein

Figure 5. Risk estimates for major adverse cardiovascular and cerebrovascular events

Forest plot displays summary estimates risk difference (RD) for cardiovascular events of angina, MACCE, coronary revascularizations

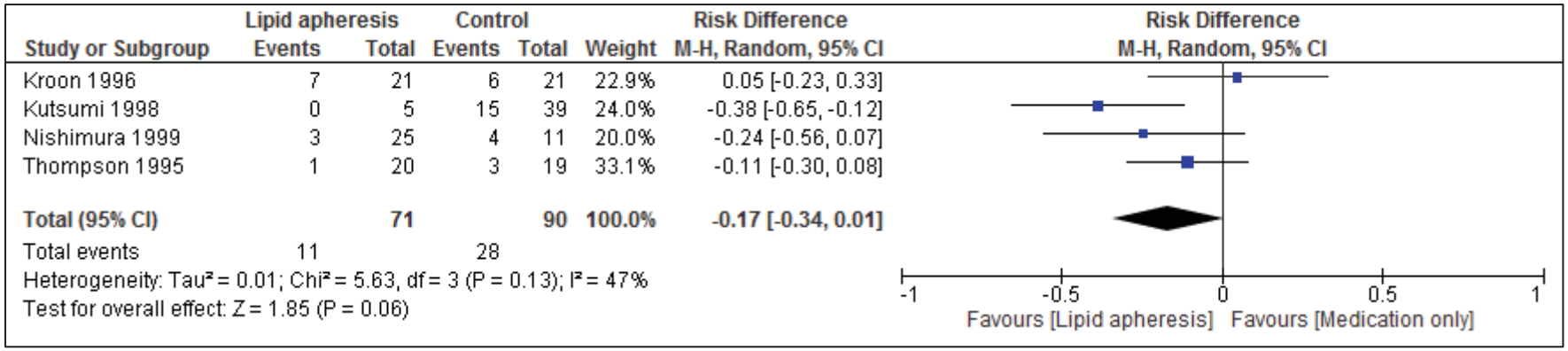


Table 1. Patient demographics. Data reported as Lipid apheresis+ drug therapy / Drug therapy alone

Study	Male (%)	Age (Yrs)	HTN (%)	DM (%)	Smoker (%)	Chol (mmol/l)	BMI (kg/m^2)	Past MI (%)	ACEi/ARB (%)	BB (%)	Aspirin/antithrombotic (%)
Matsuzaki (2002)	73/71	50/54	36/57	18/0	64/57	9.72/9.85	NR	55/29	9/29	27/29	64/57
Nishimura (1998)	68/73	51/51	12/27	12/18	32/45	7.9/8.7	21.5/21.5	48/64	NR	24/36	80/82
Kutsumi (1998)	100/80	56/61.4	80/65	40/60	NR	8.1/6.6	NR	NR	NR	60/82	100/60
Kroon (1996)	100/100	50.2/53.9	9.5/23.8	NR	14.3/19	9.72/9.85	26.6/26.2	76.2/85.7	NR	47.6/66.7	19/38.1
Thompson (1995)	70/73.6	49.2/49.8	NR	NR	5/16	9.0/8.1	27/25	35/47	NR	40/42	50/42
Daida* (1997)	88/88	57/60	NR	17/15	41/35	5.1/5.01	NR	NR	NR	NR	NR

***ACEi**; angiotensin-converting-enzyme inhibitor, **ARB**; Angiotensin II receptor blockers, **BMI**: Body mass index, **BB**; beta blocker, **Chol**; cholesterol, **DM**; Diabetes mellitus, **HTN**; Hypertension, **MI**; myocardial infarction.**NR**; not reported*

** Basic characteristics was extracted from the original study in 1994.*

Table 2. Included study characteristics. Data reported as LDL apheresis + drug therapy / Drugs alone

Study	Country	N	Design	Diagnosis of FH	QCA	LDL-A	Frequency of LDL-A	Statin	Other lipid lowering agents	Mean F/U (mths)	Primary End Point	Baseline LDL-C (mmol/l)	Baseline Lp(a) (mg/dL)	Baseline HDL (mg/dL)	Baseline TG (mg/dL)
Matsuzaki (2002)	Japan	11/7	RCT	Clinical	CARDIO-500	DSCC	2W	Pravastatin 20 mg OD or simvastatin 10 mg OD	Probucol, cholestyramine and fibrate	12	MLD	5.5/4.4	NR	33/44	143/163
Nishimura (1998)	Japan	36/11	Non-RCT	Clinical	CARDIO-500	DSCC	2W	Pravastatin 20 mg daily	Cholestyramine 27 g OD, Probucol 750 mg OD	28	MLD	6.3/6.6	NR	29/38	172/199
Kutsumi (1998)	Japan	5/39	RCT	NR	CARDIO-500	DSCC	2W	Pravastatin	NR	26	MLD	NR	NR	30/37	143/143
Kroon (1996)	Netherland	21/21	RCT	Clinical	Sun Microsystem	DSCC	2W	Simvastatin 40 mg daily	NR	24	MOD	7.78/7.85	57/38.4	35.96/35.57	89.7/102.08
Thompson (1995)	The UK	20/19	RCT	Clinical	Pie-medical, Maastricht, Netherlands	DSCC	2W	Simvastatin 40 mg daily	Cholestyramine 20 gr OD	25.2	MLD	4.15/3.4	47/34	42.5/42.5	92.08/77.3
Daida* (1997)	Japan	66/137	RCT	NR	NR	DSCC	NR	Pravastatin 10 mg daily	Niacin 1500mg OD	30	MLD	8.1/8.7	23.3/16.9	132/121	147/152

DSCC; dextran sulphate cellulose column, FH; Familial Hypercholesterolemia, FU; Follow Up, HDL; high density lipoprotein, MLD; Minimum Lumen Diameter, MOD; Minimum Obstructive Diameter, NA; Not applicable , NR;not reported, OD; daily, RCT; randomised clinical trial, TG; Triglyceride, 2W; fortnightly,
** Basic characteristics was extracted from the original study in 1994.*

Supplemental Material

The Role of Low Density Lipoprotein Apheresis in the Management of Heterozygous Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis

Contents Page

Supplementary Table S1. Search strategy

#	Searches		Results
	1	exp hypercholesterolemia/	61406
	2	exp hyperlipoproteinemia type 3/	1233
	3	exp hyperlipoproteinemia/	7639
	4	hyperlipoproteinemia type 4/	489
	5	exp hyperlipoproteinemia type 1/	771
	6	exp hyperlipoproteinemia type 5/	189
	7	dyslipidemia/	51105
	8	apolipoprotein/	9010
	9	exp hyperlipoproteinemia type 2/	7037
	10	exp low density lipoprotein receptor related protein/	2736
	11	exp apolipoprotein D/	710
	12	exp apolipoprotein A/	3442
	13	exp beta2 glycoprotein 1/	3058
	14	exp lipoprotein/	216738
	15	lipoprotein X/	307
	16	exp lipoprotein/ or exp lipoprotein A/	216738
	17	familial hyperlipemia/	828
	18	low density lipoprotein cholesterol/	77851
	19	exp very low density lipoprotein cholesterol/	5273
	20	exp pre beta high density lipoprotein/	133
	21	(High adj3 cholesterol).tw.	43215
	22	(familial adj2 hypercholesterolemia).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]	8678

23	Lp a*.mp.	11422
24	exp treatment outcome/	1227528
25	exp plasmapheresis/	32290
26	immunoadsorption/ or "isolation and purification"/	280629
27	lipid apheresis.mp.	175
28	(Lipid adj 2apheresis).ti.	0
29	Lipid-apheresis.mp.	175
30	Lipoprotein apheresis.mp.	624
31	Extracorporeal removal.mp.	240
32	Lipoprotein precipitation.mp.	85
33	Lipoprotein turnover.mp.	62
34	immunoadsorption/	3187
35	Low density lipoprotein apheresis system.mp.	5
36	Selective removal of low density lipoproteins.mp.	6
37	LDL apheresis.mp.	1133
38	LDL-apheresis.mp.	1133
39	Lp apheresis.mp.	3
40	Lipid-lowering therapy.mp.	3696
41	Low-density-lipoprotein apheresis.mp.	404
42	Lipoprotein a apheresis.mp.	16
43	Therapeutic apheresis.mp.	916
44	Apheresis with isolated lp a.mp.	0
45	random$.mp.	1354834
46	factorial$.mp.	57908

47	crossover$.mp.	82037
48	cross over$.mp.	27224
49	cross-over$.mp.	27224
50	placebo$.mp.	391753
51	(doubl$ adj blind$).mp.	217688
52	(singl$ adj blind$).mp.	36328
53	assign$.mp.	307779
54	allocat$.mp.	126292
55	volunteer$.mp.	225622
56	crossover procedure/	50551
57	double blind procedure/	136911
58	randomized controlled trial/	442655
59	single blind procedure/	26317
60	exp apheresis/ or exp lipoprotein apheresis system/	49935
61	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23	369112
62	24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 60	1551358
63	45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59	2057895
64	61 and 62 and 63	7954
65	exp animals/ not humans.sh.	22718586
66	64 not 65	105

Supplementary Table S2. Study inclusion and exclusion criteria

Trial	Main Inclusion Criteria	Main Exclusion Criteria	Primary Endpoint	Secondary Endpoint
Matsuzaki (2002)	Age of 20 -70y, LDL-C of 130 to 230 mg/dl, strict lipid-lowering diet + HMG-CoA reductase inhibitors administrated (pravastatin [20 mg/day] or simvastatin [10 mg/day]). History of cardiac events; myocardial infarction (MI), angina pectoris, asymptomatic myocardial ischemia, CABG or PTCA or with 50% stenosis of coronary artery on coronary angiogram	Pregnant women, MI or unstable angina pectoris within the previous three months, PTCA or CABG within the previous six months, Severe DM Severe HTN, Impaired hepatic or renal function, Secondary hypercholesterolemia	LDL-C	Changes in MDL measured by QCA, Coronary plaque area by IVUS
Nishimura (1998)	Tendon xanthomas, a mean of two consecutive serum total cholesterol levels ≥230 mg/dl, a mean of two consecutive serum total triglyceride levels <210 mg/dl, 8 weeks treated by a cholesterol lowering diet (equivalent to the American Heart Association Step I diet) and lipid lowering drugs. Positive family history of FH. Clinical evidence of coronary artery disease by visual assessment (>25% diameter stenosis) on the initial angiography.	Congestive heart failure, Acute MI within 1 month, PTCA within 6 months or CABG within 1 year; Severe DM, Severe renal and hepatic disease, Uncontrolled HTN, Secondary hyperlipidaemia, Medical condition that may interfere with safe LDL apheresis treatment in the judgment of the investigators	Progression and regression by the detection of MLD changes in angiography	LDL-C Clinical coronary events (unstable angina, PTCA, CABG)
Kutsumi (1998)	Significant coronary vessel disease, TC >250 mg/dl, on lipid lowering medications	Cross-over cases during observation period	Changes in the MLD measured by QCA	Cardiac events, Subjective symptoms, Plasma lipids, Cost

Kroon (1996)	A mean of two successive serum TC >8.0mmol/L (309.3 mg/dl) or LDL-C >5.8mmol/L (224.2 mg/dl) and a mean of two successive fasting serum triglyceride measurements <5.0mmol/L (193.3 mg/dl). On a standard lipid lowering diet without other lipid lowering treatments. Extensive coronary atherosclerosis as shown by visual assessment of their coronary angiogram.	Patients with a ventricular ejection fraction <0.35. AMI, PTCA, or CABG within the previous 3 months Impaired hepatic (>30% above normal range) or renal function (plasma creatinine ≥ 150μmol/L); hypertension (diastolic blood pressure ≥100mmHg), DM, Severe obesity (BMI≥30kg.m^{-2}), Homozygous FH, Secondary hyperlipidaemia, Heavy smokers (>10 cigarettes per day).	LDL-C, Average mean segment diameter and minimal obstruction diameter detected by angiography	Lipid profile ApoA1, apo B and Lp(a) measured bimonthly, Bicycle exercise tests to assess clinical symptoms and usual criteria at baseline and follow up
Thompson (1995)	Serum TC ≥8.0 mmol/L (309.3 mg/dl). Tendon xanthomata in the patient or first-degree relative, or plus either hypercholesterolemia ≥8.0 mmol/L (309.3 mg/dl) or MI before the age of 60 in a first-degree relative or before age 50 in a second-degree relative. Age of 20-64 y. At least two abnormal coronary segments (excluding total occlusions and lesions previously subjected to angioplasty) on coronary angiography.	Previous CABG, DM, Secondary hyperlipidaemia, Uncontrolled HTN (diastolic blood pressure >100 mmHg despite treatment). Premenopausal female or use of hormone-replacement therapy if postmenopausal, Partial iliac bypass	Change in mean % diameter stenosis (DS)	Change in MLD averaged over all segments. Mean MLD (mm) of worst lesion per segment (with % DS ≥20%), Serum TC, triglycerides (non-fasting), HDL-C, Apo-B, Lp(a)
Daida (1997)	Patients who underwent LDL apheresis before and after PTCA. Controls were selected randomly from patients who underwent PTCA without LDL apheresis during the same period of time	Unstable angina, AMI	TC, triglyceride, HDL-C, Lp(a)	Changes in the luminal diameter of a previously dilated segment by angiography

AMI; Acute Myocardial Infarction, Apo; apolipoprotein, , CABG; Coronary artery bypass graft surgery, DM; Diabetes Mellitus, FH; Familial hypercholesterolemia, HMG-CoA; Hepatic Hydroxymethylglutaryl-Coenzyme A, HTN; Hypertension, HDL-C; High Density Lipoprotein Cholesterol, IVUS; Intravascular ultrasound, LDL-C; Low Density Lipoprotein Cholesterol, Lp(a); Lipoprotein (a), MDL; Minimal Luminal Diameter, PTCA; Percutaneous transluminal coronary angioplasty, QCA; quantitative coronary angiogram, TC; Total Cholesterol.

Supplementary Table S3. Measurements of coronary disease by QCA

Study	Measurement of coronary artery disease
Matsuzaki (2002)	MLD; Following selection of the boundaries of a segment, the arterial borders were defined by an automated edge-detection algorithm. For calibration, the boundaries of a non-tapering part of the catheter were determined automatically over a length of approximately 2 cm.
Nishimura (1998)	MLD; Greatest narrowing and nearby proximal and distal normal diameters were measured in millimetres. Using the automated edge detecting method, identical projections of the major coronary arteries, if possible, diastolic frames were used.
Kutsumi (1998)	MLD; Measured in the proximal two segments in one vessel. The vessel that had bypass grafting and/or the target lesion by angioplasty was excluded.
Kroon (1996)	MOD; minimal obstruction diameter was used to measure localised atherosclerosis, MSD (mean segmental diameter) was used as a measure of diffuse changes. A diameter function was determined in absolute terms (in millimetres) by computing the shortest distances between the left and right contours along the vessel centreline.
Thompson (1995)	MLD; Measured (in mm) as averaged over all segments. Changes in mean % diameter stenosis (DS) measured as worst lesion in in diseased segments. The analysis was performed via Cardiovascular Angiography Analysis System
Daida (1997)	NR

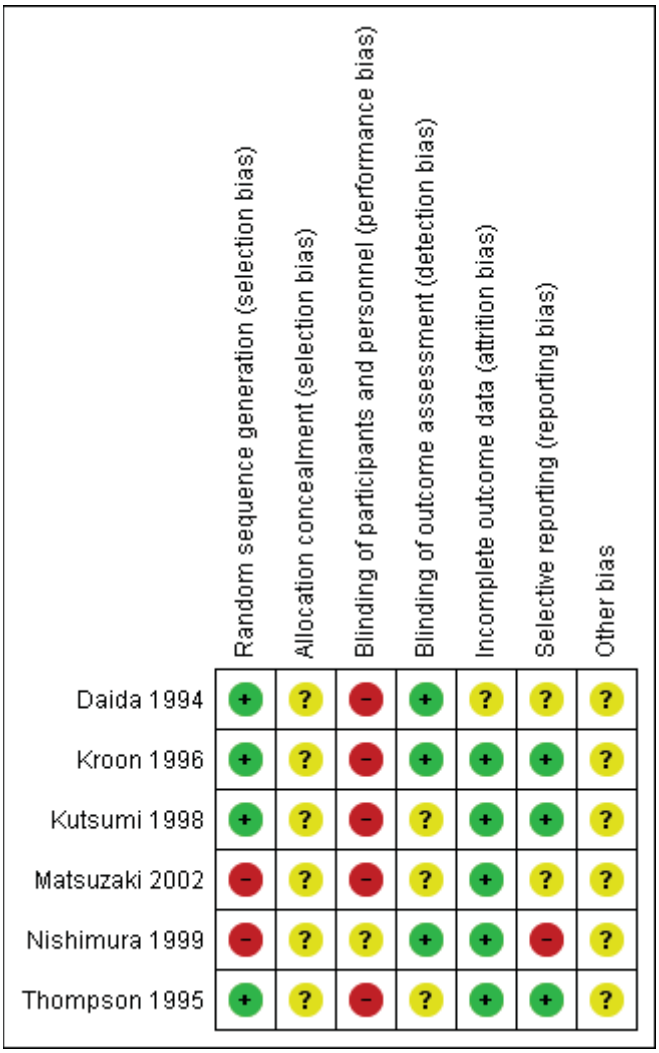
DS; diameter stenosis, MLD; Minimum Luminal Diameter, MSD; mean segment diameter,

Supplementary Table S4. Primary and secondary endpoints of the studies

Study ID	Study arms (n)	Change in MLD/MOD in LA, mm	LDL Mean ±SD (mmol/L)	Major Adverse Cardiovascular Events
atsuzaki (2002)	LA (n = 11) M (n = 7)	Segments (34) B = 1.99 ± 0.73 F = 2.11 ± 0.81 C = 0.12	B = 5.5 ± 0.6 F = 3.6 ± 0.6 C = -34.3%*	NR
Thompson (1995)	LA (n = 20) M (n = 19)	Segments (5) B = NR F = NR C = -0.004	B = 4.15 F = 1.4 C = -3.2 (0.8)	3 events (2 non-fatal MI and one PTCA) in the drug group One PTCA in the LA group Within 25.2 months
Kroon (1996)	LA (n = 21) M (n = 21)	Segments (NR) B = 1.93 ± 0.43 F = 1.92 ± 0.40 C = −0.01 ± 0.13	B = 7.78 ± 1.86 F = 2.95 ± 1.13 C = -4.83 ± 2.2	Total of 7 cardiac events in LA group 5 events in 5different patient in medical only group One TIA/stroke in medication only group
Nishimura (1998)	LA (n = 25) M (n = 11)	Segments (40) B = 1.79 ± 0.54 F = 1.99 ± 0.52 C = 0.19 ± 0.30	B = 246 ± 92 F = 140 ± 34 C = -3.2 (±1.5)	3 cardiac events in LA (1 angioplasty, 1 CABG and 1 hospital admission for UA) 4 cardiovascular events in control group (1 CABG and 3 hospital admission for UA) Within 6 months after enrolment
Kutsumi (1998)	LA (n = 5) M (n = 39)	Segments (76) B = 1.92 ± 0.54 F = 1.98 ± 0.37 C = 0.06 ± 1.21	B = 8.1 ± 3.1 F = 4.2 ± 0.7 C = NR	No patient receiving apheresis had cardiac events. 15 had angina pectoris. None of the patients had MI. in medication only group Within 26 months observation
Daida (1997)	LA (n = 66) M (n = 137)	Segments(NR) B = 1.85 ± 074 F = 2.02 ± 0.77 C = NA	B = 3.4 ± 1.18 F = 1.4 ± 0.5 C = 55 ± 10%*	NR

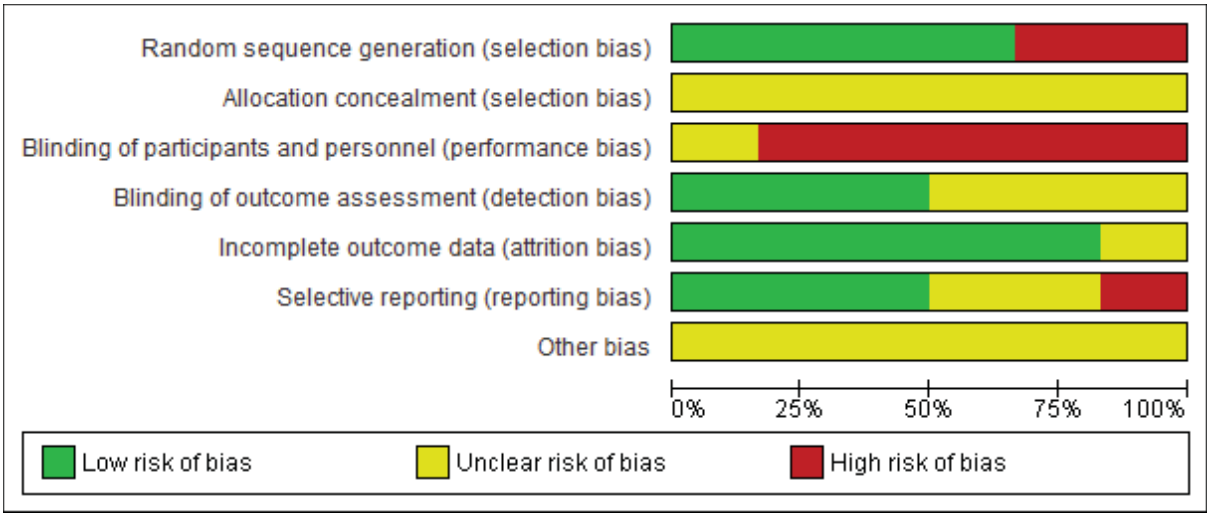
*B; Baseline, F: follow up, C; Changes, CABG; Coronary artery bypass graft surgery LA; Lipid apheresis, M; Medical therapy, MI; Myocardial infarction NR; Not Reported, PTCA; Percutaneous transluminal coronary angioplasty, TIA; Transient Ischaemic Attack, UA; Unstable angina, * reported as percentage change*

Supplementary Figure 1. (A) Individual bias assessment of included studies. **(B)** Summary bias assessment of included studies.



Caption-A

Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Caption-B

Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

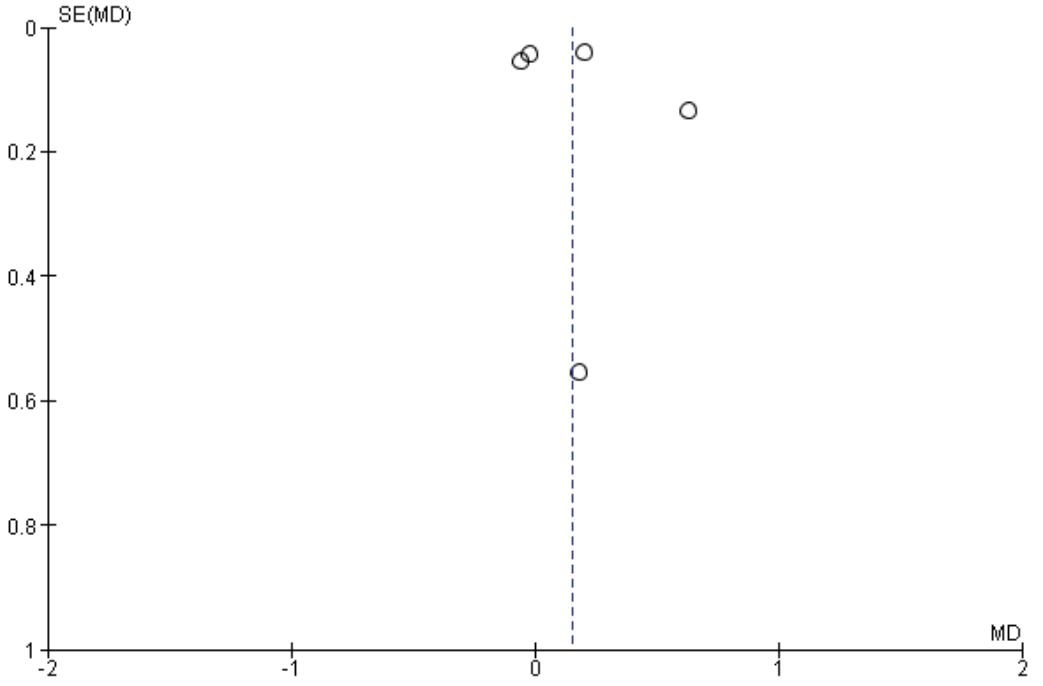
Supplementary Figure 2: Quality of the evidence (GRADE Working Group grades of assessment)

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Supplemental lipid apheresis	Lipid lowering therapy alone	Relative (95% CI)	Absolute (95% CI)		
Minimal luminal diameter measured on quantitative coronary angiography (follow up: median 24 months; assessed with: Quantitative coronary angiography)												
5	randomised trials	not serious	serious [a]	not serious	serious [b]	none	82	87	-	MD **0.15 mm more** (0.04 fewer to 0.34 more)	⨁⨁◯◯ LOW	IMPORTANT

CI: Confidence interval; **MD:** Mean difference

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio; GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

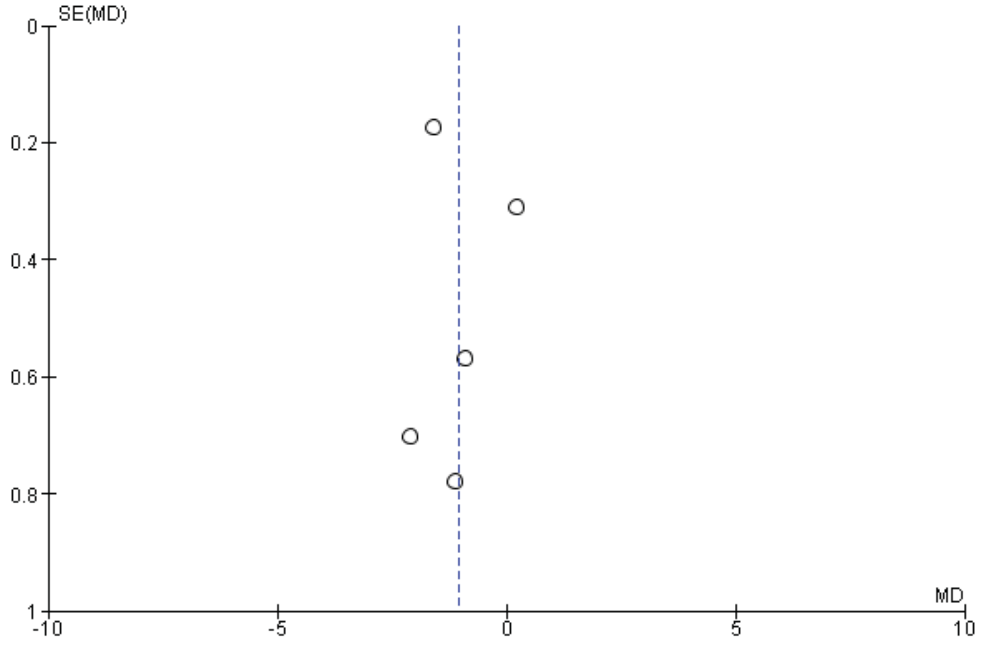
Supplementary Figure 3



Caption

Funnel plot of comparison: 1 Regression of coronary plaque volume in patients with Lipid Apheresis and lipid lowering therapy Vs Lipid lowering therapy alone, outcome: 1.1 Minimal lumen diameter (MLD) by quantitative coronary angiography [mm].

Supplementary Figure 4:



Caption

Funnel plot of comparison: 1 Regression of coronary plaque volume in patients with Lipid Apheresis and lipid lowering therapy Vs Lipid lowering therapy alone, outcome: 1.3 LDL-C response [mmol/L].

Chapter 2 C

The effect of combined ezetimibe and statin therapy versus statin therapy alone on coronary plaque volume assessed by intravascular ultrasound: A systematic review and meta-analysis

Journal of Clinical Lipidology (2018) 12, 1133–1140

Journal of Clinical Lipidology

The effect of combined ezetimibe and statin therapy versus statin therapy alone on coronary plaque volume assessed by intravascular ultrasound: A systematic review and meta-analysis

Sam Mirzaee, MD, MPH*, Paul M. Thein, MBBS, Jason Nogic, MBBS, BSci, Nitesh Nerlekar, MBBS, MPH, Arthur Nasis, MD, PhD, Adam J. Brown, MB, BChir, PhD

Monash Cardiovascular Research Centre, Monash Heart, Monash Health, Monash University, Melbourne, Australia

KEYWORDS:
Intravascular ultrasound;
Coronary atherosclerotic plaque;
Statin;
Ezetimibe therapy

BACKGROUND: Current guidelines recommend an intensive lipid-lowering therapy to achieve the low-density lipoprotein cholesterol (LDL-C) target in patients with high risk of cardiovascular disease. Former studies suggested adding ezetimibe to statin therapy in the above setting may promote plaque changes; however, this effect has not been consistently reported.

METHODS: Electronic searches were performed in MEDLINE, EMBASE, and Cochrane library on November 30, 2017 to identify prospective trials assessing the effects of combined ezetimibe and statin therapy versus statin therapy alone on atheroma volume using intravascular ultrasound. The effect size between treatment groups within individual studies was assessed by weighted mean difference (MD) using a random-effects model.

RESULTS: Eight studies were obtained for systematic review and 6 of them compromising total of 583 subjects that meet the criteria were meta-analyzed. There was a significant reduction from baseline to follow-up in total atheroma volume with an MD of –3.71 mm^3 (95% confidence interval: −5.98 to −1.44, $P < .001$), whereas analysis for percent atheroma volume demonstrated weighted MD of –0.77% (−1.68 to 0.14, $P = .10$). A substantial decrease in LDL-C was observed with MD −16.75 mg/dL (−20.89 to −12.60, $P < .00001$).

CONCLUSION: The addition of ezetimibe to statin therapy is effective in reducing total atheroma volume assessed by intravascular ultrasound and also resulted in effective reduction of plasma LDL-C levels.

Introduction

Atherosclerotic coronary artery disease (CAD) remains a leading cause of morbidity and mortality worldwide.[1] Several well-established publications indicate that risk factors accelerate the development of atheroma, with hypercholesterolemia being a primary driver for plaque

Conflict of interest: All other authors report no conflict of interest.

* Corresponding author. Dr Sam Mirzaee, Monash Cardiovascular Research Centre, Monash Heart, Monash Health, Monash University, 246 Clayton Road, Clayton, Melbourne, VIC 3168 Australia.
E-mail address: Sam.Mirzaee@Monashhealth.org

Submitted March 12, 2018. Accepted for publication June 5, 2018.

1933-2874/
https://doi.org/10.1016/j.jacl.2018.06.001

growth. A strong body of evidence, including Mendelian randomization studies, demonstrated the causality of low-density lipoprotein cholesterol (LDL-C) in CAD.[2] Furthermore, previous studies have shown a 20% reduction in 5-year incidence of adverse cardiac events per 1-mmol/L decrease in LDL-C concentration while on statin therapy.[3]

Ezetimibe is a cholesterol absorption inhibitor, exerting its effect through interaction with the Niemann-Pick C1-like protein 1 (NPC1L1) located in intestine, which leads to further reduction of circulating plasma LDL-C levels by 15–20%, when compared with statin therapy alone.[4,5] The clinical benefit of combining ezetimibe with statin therapy was confirmed in the IMPROVE-IT randomized trial, which demonstrated reduced cardiovascular events in those receiving combination therapy following presentation with an acute coronary syndrome.[6] However, the underlying mechanism of adding ezetimibe to statin therapy results in modification of cardiovascular outcome is unclear.

Atheroma (or plaque) volume is an important surrogate marker of future cardiovascular events and can be measured using a variety of imaging techniques, including intravascular ultrasound (IVUS) (Fig. 1).[7,8] Former studies using IVUS have widely recognized that statin therapy modifies the natural history of CAD by slowing plaque progression and may even result in regression of atheroma.[9,10] Although combination of ezetimibe with statins is well accepted in current cardiovascular therapeutic guidelines, the true benefits of this combination on atherosclerotic plaque in patients with existing cardiovascular disease remains to be established.[11,12]

Methods

Data sources and search strategy

The review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[13] Comprehensive searches of EMBASE, MEDLINE, and The Cochrane Library along with trial registries and abstracts from major scientific meetings were conducted to identify suitable publications. Our search was restricted to studies published in English language only. The study protocol was prospectively registered with PROSPERO (CRD42017081249) and adhered to the PRISMA guidelines. An example search strategy for EMBASE is provided in Supplemental Table 1.

Study selection

Studies analyzed are only those meeting the following inclusion criteria:

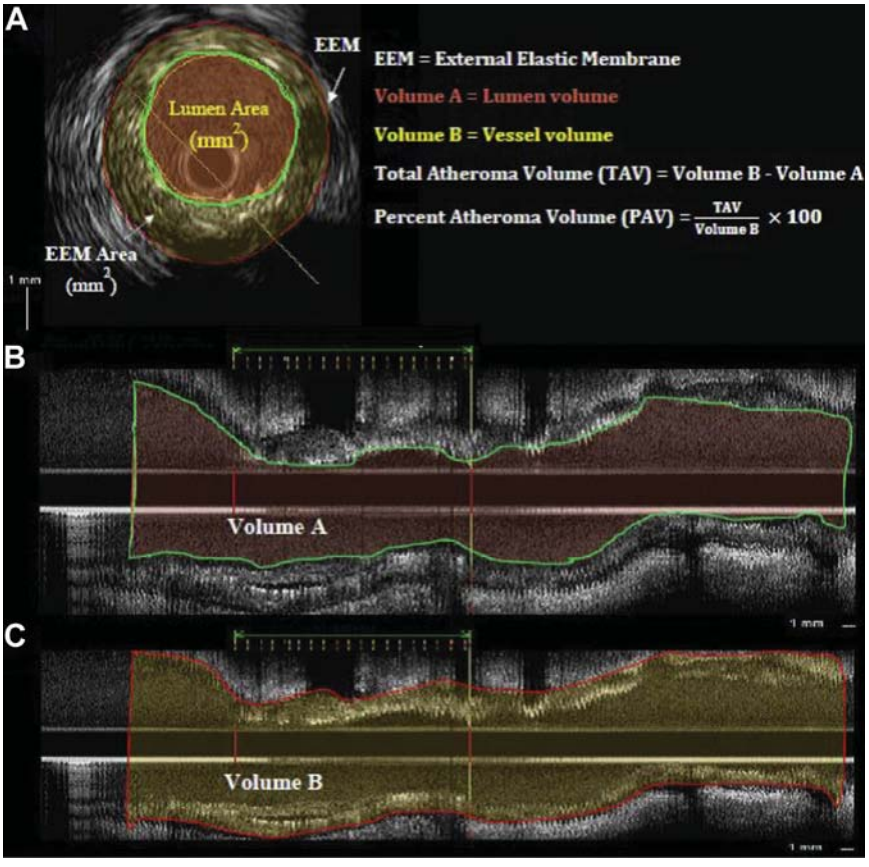


Figure 1 Intravascular ultrasound of coronary artery to assess plaque volume. (A) Planimetry of external elastic membrane (EEM) and lumen area; Formula to calculate TAV and PAV using lumen volume and vessel volume; (B) Longitudinal view of the same segment of coronary artery assessing lumen volume (Volume A); (C) Evaluation of vessel volume (Volume B). PAV, percent atheroma volume; TAV, total atheroma volume.

1. Comparisons of efficacy for a statin alone versus statin and ezetimibe combination therapy
2. Sample size of >30 patients and follow-up duration of ≥3 months
3. IVUS used as de facto modality of measuring changes in atheroma volume
4. Reporting of change in atheroma between baseline and follow-up, expressed as mean delta total atheroma volume (TAV) and/or mean delta percent atheroma volume (PAV).

The details of inclusion and exclusion criteria for each study have been summarized in Supplemental Table 2. In the event of multiple publications reporting results on the same cohort of patients, we included the most recent papers with largest sample size and follow-up period.

In this analysis, study-specific definitions of atheroma growth were used that included either changes in TAV or PAV. PAV was calculated as the proportion of total vessel wall volume occupied by atheromatous plaque, while TAV was calculated as the summation of plaque area in each measured longitudinal image, normalized to account for differences in segment length between different subjects as previously described.[14] Specific details for the calculations performed in each individual study are available in Supplemental Table 3.

Data items and collection process

Data items to be collected were predetermined before the literature search. Two authors (SM and PMT) independently conducted the search and data extraction for baseline patient demographics and clinical outcomes. Extracted data verified by the senior author (AJB) and any discrepancies resolved by consensus agreement. Within each individual trial, the risk of bias was assessed according to the Cochrane Collaboration Assessment tool (Supplemental Fig. 1).

Study endpoints

The primary endpoint of the study was defined as the change in TAV measured from baseline to follow-up by IVUS, comparing groups of subjects on statins alone versus combination of statin and ezetimibe (Supplemental Tables 4 and 5). Secondary endpoints included as changes in luminal volume and PAV on IVUS, both high-density lipoprotein cholesterol (HDL-C) and LDL-C concentrations, and systemic inflammatory activity as measured by plasma high-sensitivity C-reactive protein (hs-CRP) level (Supplemental Table 6).

Statistical analysis

The meta-analysis was performed using Review Manager (RevMan), version 5.3 (Cochrane Collaboration, Oxford, UK) and Stata 14/MP (StataCorp, TX). Outcomes were analyzed using a random-effects model and summary estimates reported as weighted mean difference (MD) with 95% confidence intervals (CI). Continuous variables are

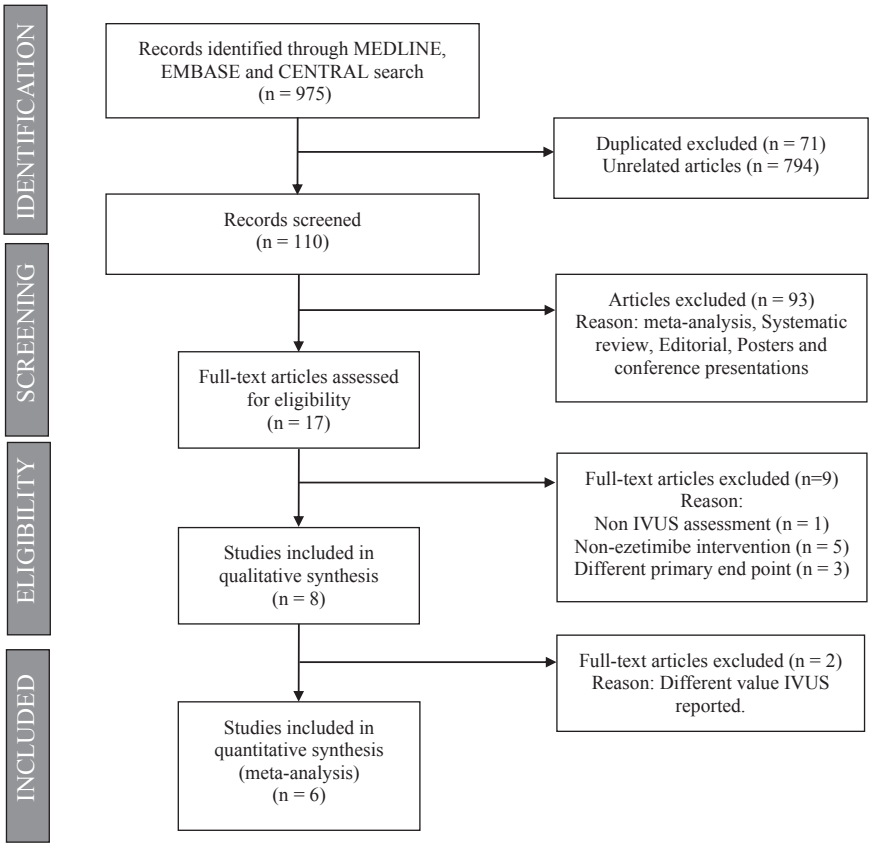


Figure 2 PRISMA flow chart of included studies. Flow chart of search yield and study selection according to PRISMA method.

reported as mean and standard deviation (SD), with nonparametric data transformed to mean (SD) as previously described.[15] Heterogeneity was assessed for all endpoints with the I^2 statistic. Heterogeneity was regarded as low, moderate, or high based on an I^2 value of 25%, 50%, and 75%, respectively.[16] For all analyses, a 2-sided P-value of $<.05$ was considered significant. The quality of outcome was assessed via Grading of Recommendations, Assessment, Development and Evaluation system with publication bias assessed visually via funnel plots and statistically by the Egger test (Supplement Figs. 2 and 4).

Results

Search results

A total of 975 initial citations were returned from the electronic searches. Following screening, 17 studies were identified for potential inclusion. Of these, 9 studies were excluded as having non-IVUS assessment (n = 1), non-ezetimibe intervention (n = 5), and different primary endpoint (n = 3). Full details of trial selection are presented in Figure 2.

Eight studies including 782 participants were included in the final quantitative analysis.[17–24] Of these, 390 (49.8%) participants received a combination of ezetimibe and statin and 392 (50.2%) received statin therapy alone. The mean age of participants is between 55–71 years, with 66–91% male and between 2–52% having diabetes mellitus. A high potency statin (either atorvastatin or rosuvastatin) was used in 7 of the included studies.[17–20,22–24] The mean duration of study follow-up was 8.81 ± 3.4 SD, ranging from 3 to 12 months from baseline. Additional baseline study and patient characteristics are presented in Tables 1 and 2, respectively.

Changes in atheroma volume

Of the 8 included studies, 6 reported the primary endpoint of change in TAV between baseline and follow-up. Treatment with combination of ezetimibe and statin therapy was associated with a significant reduction in TAV, when compared with statin therapy alone (MD –3.71 mm^3, 95% CI −5.98 to −1.44, $P < .001$) with low heterogeneity observed between studies ($I^2 = 2\%$, $P = .41$) (Fig. 3). Furthermore, sensitivity analyses were performed to assess for changes in the primary endpoint in relation to mean duration of study follow-up and the imaging resolution of IVUS catheter used (40-MHz vs 20-MHz). Although there were no differences in TAV changes by IVUS imaging resolution, studies reporting longer follow-up (>6 months) exhibited greater reduction of TAV (MD −4.74 mm^3, CI −7.88 to −1.60] vs ≤6 months (MD −2.65 mm^3, CI −5.80 to 0.51).

Nevertheless, the calculated P value for interaction was statistically insignificant ($P = .36$), which is indeed due to underpowered sample size (Supplemental Figs. 5 and 6).

Secondary endpoints

All 8 studies reported data on LDL-C concentrations, with coadministration of ezetimibe and statin being associated with a significantly lower LDL-C concentrations when compared with statin alone (MD –16.75 mg/dl, 95%

Table 1 Patient demographics

Study	Male (%)	Age (Yrs)	HTN (%)	DM (%)	Smoker (%)	FHx (%)	Chol (%)	BMI (kg/m^2)	Past MI (%)	Current MI (%)	ACEi/ARB, (%)	BB, (%)	Aspirin, (%)
Kovarnik (2012)	79/66	64/65	81/85	29/28	68/62	54/43	73/72	NR	49/38	NR	75/66	70/71	NR
Nakajima (2014)	80/84	64/61	70/69	32/40	64/69	24/31	NR	24/25	NR	66/64	68/62	48/44	100/100
Masuda (2015)	91/84	64/70	62/90	52/42	43/21	48/32	NR	25/24	24/21	NR	10/16	43/47	NR
Tsujita (2015)	78/78	66/67	75/66	29/30	20/32	NR	72/69	25/25	15/13	61/65	73/63	41/50	100/100
Wang (2015)	72/73	63/65	50/48	36/35	62/60	NR	NR	NR	NR	56/57	36/33	78/73	100/100
Hougaard (2016)	91/82	55/57	16/18	2/2	58/52	44/50[#]	74/68	27/27*	NR	100/100	9/9	0/5	NR
Lee (2016)	79/75	61/59	50/58	32/25	44/50	NR	NR	NR	NR	100/100	88/92	91/89	100/100
Ueda (2017)	76/81	71/68	85/80	37/31	33/41	NR	NR	25/24	13/30	NR	NR	NR	96/98

Data reported as Ezetimibe + Statin/Statin alone.

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, beta blockers; BMI, body mass index; Chol, hypercholesterolemia; DM, diabetes mellitus; FHx, family history; HTN, hypertension; MI, myocardial infarction; NR, not reported.

[#][refer to family history of myocardial infarction or familial hyperlipidemia ()].

*Data reported as median.

Table 2 Included study characteristics

Study	Country	Population	IVUS Catheter	Method of IVUS analysis	Patients (n)	Statin	F/U (mths)	Design	Primary IVUS endpoint	Baseline TC (mg/dL)	Baseline LDL (mg/dL)	Baseline HDL (mg/dL)	Baseline TG (mg/dL)
Kovarnik (2012)	Czech Republic	SAP	Eagle Eye	Virtual histology	42/47	Atorvastatin	12	Single-blinded RCT	Plaque composition	193.3/177.9	119.9/104.4	46.4/46.4	65.7/65.7
Nakajima (2014)	Japan	ACS	Atlantis SR Pro 2	Volumetric IVUS	50/45	Atorvastatin	6	Open label nonrandomized	Plaque volume	NR	116.2/114.3	50.4/45.5	106.3/117.5
Tsujita (2015)	Japan	ACS/SAP	NR	Volumetric IVUS	100/102	Atorvastatin	9–12	Single-blinded RCT	% Atheroma volume	177.3/172.7	109.8/108.3	41.1/40	114/116
Masuda (2015)	Japan	SAP	Atlantis SR Pro 2	Volumetric IVUS	21/19	Rosuvastatin	6	Open-label RCT	Plaque volume	204.4/194	131.8/123	53.1/47.1	129.7/144.9
Wang (2015)	China	ACS/SAP	Eagle Eye	Virtual histology	50/48	Rosuvastatin	12	RCT	EEM Area/plaque burden	220.4/216.6	139.2/135.3	42.5/42.5	77.3/73.4
Hougaard (2016)	Denmark	ACS	Atlantis SR Pro	Volumetric IVUS	43/44	Atorvastatin	12	Double-blinded RCT	Relative NC content	204.9/220.4	143.1/158.5	42.5/42.5	NR
Lee (2016)	Korea	ACS	Eagle Eye	Virtual histology	34/36	Simvastatin/ Pravastatin	3	Open-label RCT	Plaque composition in NCL	190.8/196.8	111.4/119.1	36/39.4	120.6/136.8
Ueda (2017)	Japan	SAP	Atlantis SR Pro 2	Volumetric IVUS	54/54	Atorvastatin	9	Open-label RCT	Plaque volume	168/162	101/101	47/45	114/113

Data reported as Ezetimibe + Statin/Statin alone.

EEM, external elastic membrane; F/U, follow-up; HDL, high-density lipoprotein; IVUS, intravascular ultra sound; NC, necrotic core; NR, not reported; NCL, nonculprit lesion; LDL, low-density lipoprotein; RCT, randomized control trial; SAP, stable angina pectoris; TC, total cholesterol; TG, triglycerides.

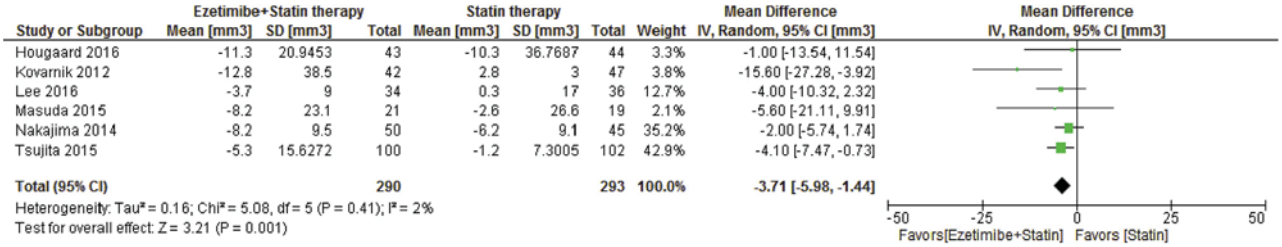


Figure 3 Forest plot of comparison showing change in TAV assessed by IVUS (mm^3). Forest plot showing the mean difference of TAV changes in coronary arteries assessed by IVUS between 2 groups of participants who received combination therapy (ezetimibe + statin) versus statin only. IVUS, intravascular ultrasound; TAV, total atheroma volume.

CI −20.89 to −12.60, $P < .001$, $I^2 = 42\%$) (Fig. 4A). However, there were no significant differences in concentrations of HDL-C (MD 0.31 mg/dl, 95% CI −1.38 to 1.99, $P = .72$, $I^2 = 0\%$) or hs-CRP levels between treatment groups (MD −0.01 mg/dl, 95% CI −0.09 to 0.06, $P = .68$, $I^2 = 89\%$) (Fig. 4B and C). Meta-regression to assess the correlation between LDL-C reduction and TAV changes was not performed due to substantial heterogeneity among studies. For the meta-analysis on PAV change, we added a recently published study showing PAV outcome

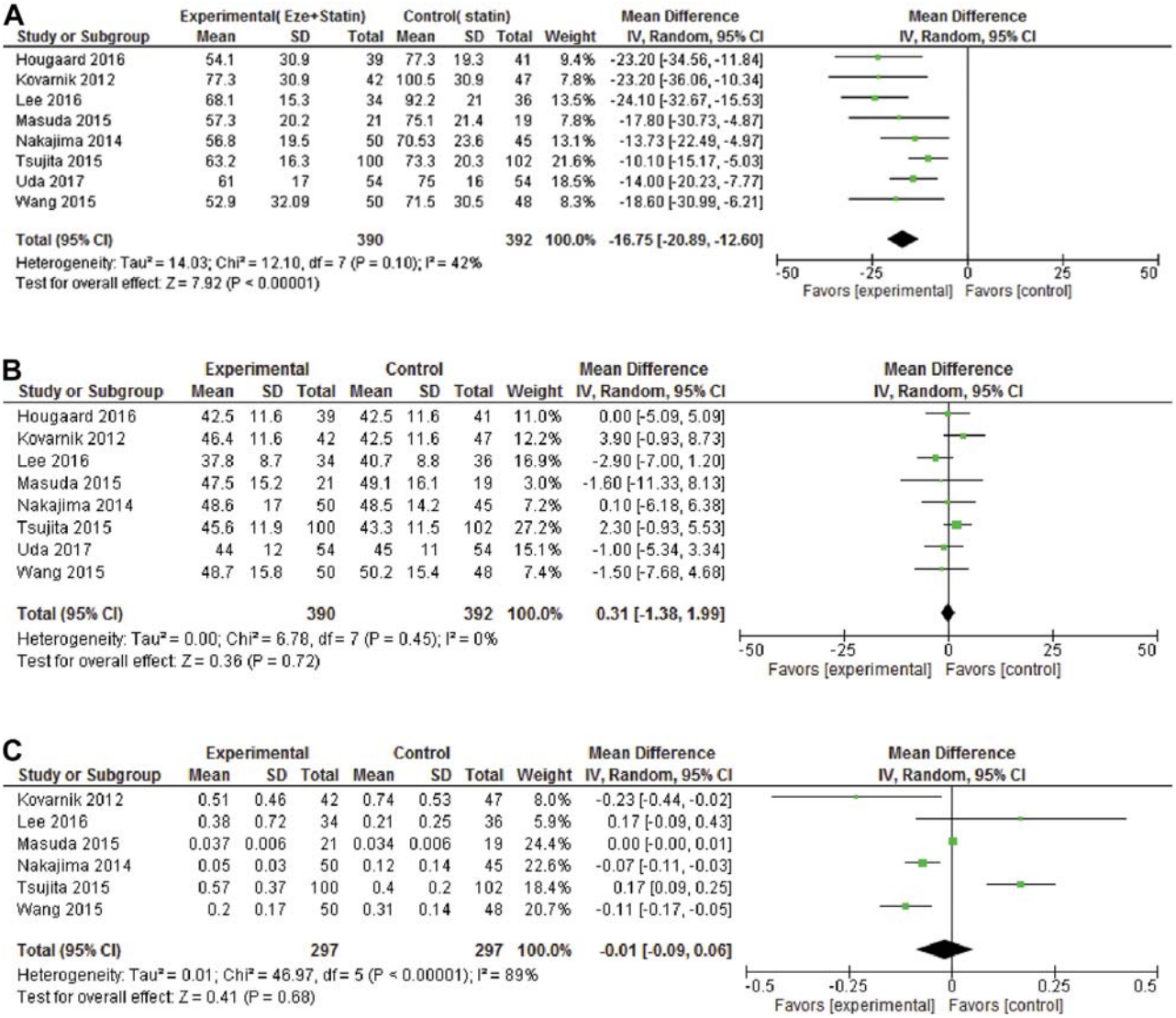


Figure 4 Forest plot showing change in (A) LDL-C between groups [mg/dL], (B) HDL between groups [mg/dL], and (C) hs-CRP between groups [mg/dL]. Forest plot showing the effect of combined therapy with ezetimibe and statin versus statin only on plasma biomarkers include LDL-C, HDL-C, and hs-CRP. hs-CRP, high sensitivity C reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

for statin plus ezetimibe versus statin alone (Supplemental Fig. 7).[25] Finally, we observed no statistically significant changes in PAV and luminal volume, as assessed by IVUS, with combination of ezetimibe and statin therapy (MD of −0.77%, −1.68 to 0.14, $P = .10$, $I^2 = 12\%$ and MD of −0.02 mm^3, −0.09 to 0.06, $P = .68$, $I^2 = 90\%$, respectively) (Supplemental Figs. 7 and 8).

Publication bias

Although there was no statistically significant publication bias, as assessed by Egger test ($P = .49$), the funnel plots were not symmetrically distributed visually; thus, publication bias cannot be excluded.

Discussion

To our knowledge, this is the first meta-analysis to demonstrate that use of combination of statin and ezetimibe therapy is associated with a significantly greater reduction in TAV, when compared with lone statin therapy. The extent of TAV reduction appeared to be temporal, with greater decreases in plaque volume found in studies reporting outcome beyond 6 months. In addition, we find that combination therapy was associated with much greater reductions in circulating LDL-C levels, but no clear changes in either HDL-C or hs-CRP between treatment groups.

When ezetimibe therapy is combined with a statin, it produces an additive effect, improving the lipid profile markedly.[26,27] This is again reflected in our data, where combination therapy was associated with significant reductions in circulating LDL-C levels. In this review, the cardiovascular benefits of ezetimibe and statin combination therapy was observed solely in association with LDL-C reduction, suggesting we should not lose sight of lipid hypothesis process as the lead to development of atherosclerosis.[28] Yet, due to heterogeneous subjects, the potential difference in basic conventional risk factors or unmeasured confounding elements, the pleiotropic effect of this combination cannot be entirely excluded.

Notably, the cardiovascular effects of combined ezetimibe and statin therapy have been more enhanced in high-risk subgroups such as diabetes even with modest LDL-C elevation.[6,29] This perhaps raise a potential unrestricted benefits of adding ezetimibe for postacute coronary syndrome patients. The main focus of current therapeutic guidelines is to achieve recommended target LDL-C,[30] but variation in lipid-lowering regimen in conjunction with basic characteristics of subjects may also have substantial impact on the cardiovascular outcomes. Hence, there is a need for large clinical trials of treating to target LDL-C, non–HDL-C, and apolipoprotein-B (APO-B) as primary endpoints using a scheme of drug therapy employing as many as agents as necessary to achieve goal lipid levels using agents known to later atherosclerosis progression of able to induce regression or impact event rates. At minimum, this list of candidate medications would include statins, ezetimibe, PCSK9 monoclonal antibodies, and bile acid sequestrants.

Study limitations

These results must be interpreted in view of their limitations. First, the heterogeneity of type of HMG-CoA reductase inhibitor used in each trial may have impacted the results and formal sensitivity analysis was not possible as there was only one study that used low intensity statins. In addition, statin dosage was diverse, which may impact on the endpoints. Second, IVUS was completed in only one coronary vessel, which may not reflect changes in TAV throughout the coronary tree. Finally, there was no consistency among the studies in terms of the time interval between pretreatment and posttreatment IVUS and LDL-C measurements, and this may have impacted on the degree of plaque and LDL-C change.

Conclusions

Our data suggest the addition of ezetimibe to statin therapy results in significantly increased regression of TAV and lowering of plasma LDL-C when compared with statin therapy alone.

Acknowledgments

SM has received consultancy fees from AMGEN and Merck. AJB is supported by a School of Clinical Sciences at Monash Health, Monash University Early Career Practitioner Fellowship award. NN is supported by National Health and Medical Research Council of Australian Government research grant.

Authors' contributions: SM contributed to study conception and design; PMT and JN contributed to acquisition of data; SM, PMT, and NN analyzed and interpreted the data; SM drafted the article; AN and AJB made the critical revision. All authors have read and given final approval of the final version of the article.

References

1. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics–2008 update: a report from the American heart association statistics committee and stroke statistics subcommittee. *Circulation*. 2008; 117(4):e25–e146.
2. Okuyama H, Hamazaki T, Hama R, et al. A critical review of the consensus statement from the european atherosclerosis society consensus panel 2017. *Pharmacology*. 2018;101(3–4):184–218.
3. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet*. 2005;366(9493):1267–1278.
4. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of

coronary atherosclerosis: a randomized controlled trial. *JAMA*. 2004; 291(9):1071–1080.
5. Lin X, Racette SB, Ma L, Wallendorf M, Ostlund RE Jr. Ezetimibe increases endogenous cholesterol excretion in humans. *Arterioscler Thromb Vasc Biol*. 2017;37(5):990–996.
6. Giugliano RP, Cannon CP, Blazing MA, et al. Benefit of adding ezetimibe to statin therapy on cardiovascular outcomes and safety in patients with vs. without diabetes: results from IMPROVE-IT. *Circulation*. 2018;137:1571–1582.
7. Dohi T, Miyauchi K, Okazaki S, et al. Plaque regression determined by intravascular ultrasound predicts long-term outcomes of patients with acute coronary syndrome. *J Atheroscler Thromb*. 2011;18(3): 231–239.
8. Puri R, Nissen SE, Shao M, et al. Coronary atheroma volume and cardiovascular events during maximally intensive statin therapy. *Eur Heart J*. 2013;34(41):3182–3190.
9. Kaneda H, Ako J, Terashima M. Intravascular ultrasound imaging for assessing regression and progression in coronary artery disease. *Am J Cardiol*. 2010;106(12):1735–1746.
10. Tomey MI, Narula J, Kovacic JC. Advances in the understanding of plaque composition and treatment options: year in review. *J Am Coll Cardiol*. 2014;63(16):1604–1616.
11. Wang J, Ai XB, Wang F, Zou YW, Li L, Yi XL. Efficacy of ezetimibe combined with atorvastatin in the treatment of carotid artery plaque in patients with type 2 diabetes mellitus complicated with coronary heart disease. *Int Angiol*. 2017;36(5):467–473.
12. Kastelein JJ, Sager PT, de Groot E, Veltri E. Comparison of ezetimibe plus simvastatin versus simvastatin monotherapy on atherosclerosis progression in familial hypercholesterolemia. Design and rationale of the ezetimibe and simvastatin in hypercholesterolemia enhances atherosclerosis regression (ENHANCE) trial. *Am Heart J*. 2005; 149(2):234–239.
13. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7):e1000097.
14. Nicholls SJ, Hsu A, Wolski K, et al. Intravascular Ultrasound-Derived Measures of Coronary Atherosclerotic Plaque Burden and Clinical Outcome. *J Am Coll Cardiol*. 2010;55(21):2399–2407.
15. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol*. 2005;5:13.
16. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557–560.
17. Ueda Y, Hiro T, Hirayama A, et al. Effect of ezetimibe on stabilization and regression of intracoronary plaque- The ZIPANGU study. *Circ J*. 2017;81(11):1611–1619.
18. Wang X, Zhao X, Li L, Yao H, Jiang Y, Zhang J. Effects of combination of ezetimibe and rosuvastatin on coronary artery plaque in patients with coronary heart disease. *Heart Lung Circ*. 2016;25(5): 459–465.
19. Kovarnik T, Mintz GS, Skalicka H, et al. Virtual histology evaluation of atherosclerosis regression during atorvastatin and ezetimibe administration: HEAVEN study. *Circ J*. 2012;76(1):176–183.
20. Hougaard M, Hansen HS, Thayssen P, et al. Influence of ezetimibe in addition to high-dose atorvastatin therapy on plaque composition in patients with ST-segment elevation myocardial infarction assessed by serial: Intravascular ultrasound with iMap: the OCTIVUS trial. *Cardiovasc Revasc Med*. 2017;18(2):110–117.
21. Lee J-H, Shin D-H, Kim B-K, et al. Early effects of intensive lipid-lowering treatment on plaque characteristics assessed by virtual histology intravascular ultrasound. *Yonsei Med J*. 2016;57(5):1087–1094.
22. Tsujita K, Sugiyama S, Sumida H, et al. Impact of dual lipid-lowering strategy with ezetimibe and atorvastatin on coronary plaque regression in patients with percutaneous coronary intervention: the multicenter randomized controlled precise-ivus trial. *J Am Coll Cardiol*. 2015; 66(5):495–507.
23. Masuda J, Tanigawa T, Yamada T, et al. Effect of combination therapy of ezetimibe and rosuvastatin on regression of coronary atherosclerosis in patients with coronary artery disease. *Int Heart J*. 2015; 56(3):278–285.
24. Nakajima N, Miyauchi K, Yokoyama T, et al. Effect of combination of ezetimibe and a statin on coronary plaque regression in patients with acute coronary syndrome: ZEUS trial (eZEtimibe Ultrasound Study). *IJC Metab Endocr*. 2014;3:8–13.
25. Hibi K, Sonoda S, Kawasaki M, et al. Effects of ezetimibe-statin combination therapy on coronary atherosclerosis in acute coronary syndrome. *Circ J*. 2018;82(3):757–766.
26. Kater A-LA, Batista MC, Ferreira SR. Synergistic effect of simvastatin and ezetimibe on lipid and pro-inflammatory profiles in prediabetic subjects. *Diabetology Metab Syndr*. 2010;2(1):34.
27. Phan BAP, Dayspring TD, Toth PP. Ezetimibe therapy: mechanism of action and clinical update. *Vasc Health Risk Management*. 2012;8: 415–427.
28. Gotto AM. Jeremiah metzger lecture: cholesterol, inflammation and atherosclerotic cardiovascular disease: is it all LDL? *Trans Am Clin Climatol Assoc*. 2011;122:256–289.
29. Blazing MA, Giugliano RP, de Lemos JA, et al. On-treatment analysis of the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT). *Am Heart J*. 2016;182:89–96.
30. Nicholls SJ, Puri R, Anderson T, et al. Effect of evolocumab on progression of coronary disease in statin-treated patients: The glagov randomized clinical trial. *JAMA*. 2016;316(22):2373–2384.

Table S1 EMBASE search strategy

No.	Search	Results
1	Hydroxymethylglutaryl-CoA reductase inhibitors/	54,934
2	exp Ezetimibe/	8033
3	exp Ezetimibe, simvastatin drug combination/	1215
4	Anticholesteremic agents/	8073
5	Hypolipidemic agents/	25,729
6	exp Plaque, atherosclerotic/	28,928
7	exp Coronary artery disease/	282,000
8	(Coronary adj3 plaque*).mp.	6855
9	(Coronary adj3 athero*).mp.	32,633
10	Coronary plaque burden.mp.	290
11	exp Ultrasonography, Interventional/	899
12	(Intravascular adj2 ultrasound*).mp.	15,215
13	Intravascular ultrasound.mp.	15,160
14	1 or 2 or 3 or 4 or 5	87,518
15	6 or 7 or 8 or 9 or 10	308,558
16	11 or 12 or 13	16,078
17	14 and 15 and 16	702

exp, explode.

Table S2 Study inclusion and exclusion criteria

Trial	Main inclusion criteria	Main exclusion criteria	Primary endpoint	Secondary endpoint
OCTIVUS Hougaard (2016)	First-time STEMI; no prior treatment with statins or other lipid-lowering drugs; and a nonsignificant lesion in one of the 2 nonculprit coronary arteries (angiographic diameter stenosis >20% and <50%)	Age <18 or > 81 y; serum creatinine >176 μmol/L; women with child-bearing potential who were not using chemical or mechanical contraception; history of malignancy unless a disease-free period of more than 5 y present; participation in another randomized trial; and treatment with cyclosporine or fibrates	Change in the relative necrotic core content after 12 mo	Change in FT, LT, and CT plaque volume together with changes in TAV and PAV
HEAVEN Kovarnik	Stable angina with native artery stenosis of 20–50% on angiography with no indication for PCI or CABG; plaque length >20 mm on IVUS (plaque length = an artery segment with plaque burden >20% by IVUS)	Significant stenosis found during angiographic assessment(>50% stenosis) or 20–50% stenosis on angiography with indication for PCI or CABG or plaque length on IVUS equal to or less than 20 mm	Change in plaque composition during 2 types of lipid-lowering therapy	Change in plaque, lumen, and vessel volumes and change in lipids and proinflammatory cytokines with a known relationship to CAD
Lee	At least 20 y old at the clinical presentation of AACS and has de novo lesions with diameter stenosis <50% by visual estimation, which were located in nonculprit vessels; reference vessel diameter was >3.0 mm and the segment length of 10–20 mm	Failed PCI of culprit lesions; a candidate for CABG, in cardiogenic shock; history of use of lipid-lowering agents before enrollment; significant hepatic dysfunction (>=3 the normal reference values); significant renal dysfunction (serum Cr > 2.0 mg/dL); significant leukopenia, thrombocytopenia, anemia, or known bleeding diathesis; pregnant or potentially child-bearing; and saphenous vein graft lesions	Quantitative and qualitative changes in plaque components of nonculprit lesions in patients with ACS	Correlation between the changes in LDL-C levels and changes in the absolute volume of plaque components

(*continued on next page*)

Table S2 (*continued*)

Trial	Main inclusion criteria	Main exclusion criteria	Primary endpoint	Secondary endpoint
Masuda	Aged 20 to 80 y with clinically stable angina pectoris; undergoing elective PCI for at least 1 significant obstructive lesion with > 1 untouched nonculprit target lesion for imaging with <50% luminal diameter narrowing that could be clearly imaged by IVUS; LDL-C level >2.6 mmol/L (100 mg/dL) at entry, regardless of prior statin use	History of ACS within 3 mo prior to entry; heart failure (NYHA class III or IV); secondary CTO, uncontrolled hypertension (BP > 200/110 mmHg despite therapy), uncontrolled DM (HbA1C >= 9.5%); persistent liver dysfunction with >= 2 upper limit of normal (ULN) of serum transaminase; serum Cr > 2.0 mg/dL or Cr clearance of <30 mL/min; unexplained serum CK level of >3 times the normal reference values; history of allergy/sensitivity reaction to any statin and/or ezetimibe; taking rosuvastatin or ezetimibe before enrollment; or recommended CABG	Change in the % PV during the observation period	Nominal change in percent PV (%PV) and nominal change in normalized PV (follow-up values minus their baseline values)
Nakajima	ACS and 20 to <79 y old who had emergency PCI in a culprit lesion and untouched nonculprit target lesion of <25% stenosis that could be imaged by IVUS	Failed PCI; recommended CABG; administration of lipid-lowering drugs (statin/fibrate/probucol/analog, nicotinic acid, or other prohibited drugs) before enrollment; renal failure (Scr >2.0 mg/dL), moderate-severe heart failure, diseased bypass graft, and left main coronary artery occlusion of >50%.	The % change in coronary PV during the observation period.	Major adverse events were defined as all-cause death, nonfatal ACS, target vessel revascularization, and stroke

(*continued on next page*)

Table S2 (*continued*)

Trial	Main inclusion criteria	Main exclusion criteria	Primary endpoint	Secondary endpoint
PRECISE-IVUS Tsujita	Signed written informed consent; 30 to 85 y old; plan to undergo PCI and LDL-C >= 100 mg/dL	Familial hypercholesterolemia; being treated with Zetia (Ezetimibe); being treated with Fibrates; renal insufficiency (serum creatinine >= 2.0 mg/dl); altered hepatic function (serum aspartate aminotransferase or alanine aminotransferase >= 3-folds of standard value in each institute); undergoing hemodialysis or peritoneal dialysis; allergic to Lipitor and/or Zetia; severe underlying disease; lack of decision-making capacity; recognized as inadequate by attending doctor	Absolute change from baseline to follow-up in percent atheroma volume (PAV) in the target lesion	Percentage change in atheroma volume, MLD and (%DS) and serum lipids; correlation between regression of coronary plaque and serum lipids profiles/ inflammatory markers; changes in hs-CRP, PV of PCI target lesion, and MLD/%DS of PCI target lesion; MACE; all-cause death
Wang	One or more atherosclerotic lesions near mid coronary arteries on QCA (borderline lesions = 40–70% stenosis, severe lesions >75% stenosis); TC >= 5.2 mmol/L and/or LDL >= 3.6 mmol/L	Contraindications for the intervention; statin use contraindicated (active hepatitis); high transaminase levels (>2-fold normal)	New or recurrence MI, unstable angina pectoris, cardiac death orc stroke	Changes in blood lipid levels, high-sensitivity CRP (hs-CRP), interleukin-6 (IL-6), and matrix metalloproteinase- 9 (MMP-9) at baseline and follow-up.
ZAPINGU Ueda	Stable coronary artery disease undergoing elective PCI and having yellow color plaque in IVUS	No adequate IVUS data. No yellow plaque of grade ≥ 2	Changes in % plaque volume and plaque color in 9 mo follow-up	Changes in laboratory data, lipid biomarkers and hs-CRP

ACS, acute coronary syndromes; CAD, coronary artery disease; CT, calcific plaque; CTO, chronic total occlusion; Cr, serum creatinine; DM, diabetes mellitus; FT, fibrotic plaque; LDL-C, low-density lipoprotein cholesterol; LT, lipidic plaque; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac events; MI, myocardial infarction; MLD, minimal luminal diameter; NYHA, New York heart association; PCI, percutaneous coronary intervention; PV, plaque volume; SAP= stable angina pectoris; STEMI, ST-segment elevation myocardial infarction; TC, total cholesterol; %DS, percent diameter stenosis; %, percent.

Table S3 TAV, PV, and PAV definitions of individual studies

Study	Measurement of atheroma burden
Hougaard 2016	TAV = Vessel volume minus lumen volume PAV=(TAV/vessel volume) × 100%
Kovarnik 2012	Indexed TAV (TAVi) = Σ(EEMCSA−lumenCSA)/plaque length Change in TAVi: $TAVi_{follow\text{-}up}$–$TAVi_{baseline}$ PAV = [Σ(EEMCSA − lumenCSA)/Σ EEMCSA] × 100 Change in PAV: $PAV_{follow\text{-}up}$–$PAV_{baseline}$
Lee 2016	Plaque plus media (or atheroma) CSA= The EEM CSA minus the lumen CSA Plaque (or atheroma) burden = Plaque plus media CSA divided by the EEM CSA.
Masuda 2015	PV = Σ (EEMCSA-lumenCSA) %PV = Percent change in PV (follow-up values minus their baseline values) L-median = the median value of the analyzed length in all subjects L-measured = the analyzed length of each plaque.
Nakajima 2014	PV = the sum of the differences between the external elastic membrane (EEM) and lumen area across all evaluated frames. % PV = the change in PV (follow-up minus baseline PV) divided by the baseline PV
Tsujita 2015	PAV = [Σ(EEMCSA−lumenCSA)/Σ EEMCSA] × 100 $TAV_{normalized}$ = Σ(EEMCSA−lumenCSA)/no: of analyzed frames per patients × median number of analyzed frames in the population
Wang 2015	Plaque burden = Area stenosis rate (MLA/EEM × 100%)
Ueda 2017	Percentage plaque volume, Yellow color grade

EEMCSA, external elastic membrane cross-sectional area; Lumen CSA, luminal cross-sectional area; PAV, percent atheroma volume; PV, plaque volume; TAV, total atheroma volume.

Table S4 IVUS definitions

Study ID	IVUS catheter	Pullback	Site of pullback
Hougaard 2016	Mechanical 40 MHz Atlantis SR Pro IVUS catheter (Both Boston Scientific, USA)	Automatic, speed 0.5 mm/s from distally to an angiographic insignificant lesion to aorta	Studied 10 mm most diseased segment, from distally to an angiographically insignificant lesion to the aorta
Kovarnik 2012	Phased array probe (Eagle Eye 20 MHz 2.9 F monorail) (research pullback, model R-100 made by Volcano Corporation, Rancho Cordova, CA, USA)	Automatic pullback, Speed 0.5 mm/s, probe introduced into selected CA beyond a distal fiduciary point (FP) (well-defined side branch) to the proximal FP at the left main bifurcation in the left CA* and the first branch or well-defined calcification in right coronary artery (same FPs of the same CA* at 12 mo)	The artery with the longer plaque was selected if $>$ 1 CA eligible
Lee 2016	2.9 F catheter (Eagle Eye 20 MHz phased array, Volcano Corp, Rancho Cordova, CA, USA)	Pullback speed 0.5 mm/s	10 mm segment (centered on the segment with minimal lumen area)
Masuda 2015	2.6 F catheter (Atlantis SR Pro2, 40 MHz, Boston Scientific, Marlborough, MA, USA)	Motorized pullback at 0.5 mm/s, Transducer positioned as distal as possible to the target lesion and withdrawn, the same coronary artery segment assessed under identical conditions to those at baseline	Target plaque lesion = non-PCI site ($>$5 mm proximal or distal to PCI site or lesions of untreated vessel) with $<$50% angiographic LD narrowing
Nakajima 2014	Atlantis SR Pro2, Boston Scientific, 40-MHz catheter, Natick, USA	Motorized pullback at 0.5 mm/s	Target segment = A single lesion in a non-PCI site with a reproducible index side branch on the PCI vessel
Tsujita 2015	40–45 MHz mechanical rotation, Boston Scientific, Natick, MA, USA; Terumo Corp., Tokyo, Japan; Volcano Corp., Rancho Cordova, CA, USA	Automatic pullback, Speed 0.5 mm/s, IVUS catheter was advanced into a PCI or non-PCI vessel as far distally possible to safely reach to obtain the longest possible target segment for analysis, and it was then withdrawn	Target segment = a non-PCI site ($>$5 mm proximal or distal to the PCI site) with a reproducible fiduciary index, usually a side branch, as the beginning and endpoint of the segments to be analyzed
Wang 2015	Phased array, 3.2 F catheter (Eagle Eye; 20 MHz Volcano Corp., San Diego, CA, USA)	Speed 0.5 mm/s, Probe in the distal end of stenotic lesion first then moved to proximal end	One or more atherosclerotic lesions near mid coronary arteries on QCA (borderline lesions = 40–70% stenosis, severe lesions $>$75% stenosis); TC $\geq$ 5.2 mmol/L and/or LDL-C $\geq$ 3.6 mmol/L

CA*, coronary artery; F, French; FP, fiduciary point; IVUS, intravascular imaging ultrasound; LD, luminal diameter; LDL-C, low-density lipoprotein cholesterol; MHz, Megahertz; PCI, percutaneous coronary intervention; QCA, quantitative coronary angiogram; TC, total cholesterol.

Table S5 Primary clinical endpoints of the studies

Study ID	Study arms, n	Change in TAV, mm^3	Lumen volume, mm^3	LDL Mean ± SD
Hougaard 2016	E 43	Entire pullback(34.4 ± 9 mm) B = 200 (135.6, 311.9) F = 189.3(126.4, 269.1) C= −11.3 (−25.5, −2.4) *P* < .001 Most diseased 10 mm B = 67.4 (48.3, 95.2) F = 60 (42.2, 82) C = −4 (−11.6, 2.4)	Entire pullback B = 311.5 (236.9, 391.7) F = 293.2 (213.7, 372.1) C = −12 (−29.2, 4.3) P 0.009 10 mm B = 81.5 (61.2, 105.5) F = 83.1 (65.3, 108.6) C = −0.9 (−7.7, 5.6) P 0.72	B = 3.7 ± 0.7 F = 1.4 ± 0.8 C = −2.3 ± 0.9
	S 44	Entire pullback B = 218.4(163.5, 307.9) F = 212.2(149.9, 394.8) C = −10.3 (−28.6, 9.8) *P* = .07 10 mm B = 74.5 (54.9, 99.6) F = 68 (44.9, 93.6) C = −5.3 (−14.6, 42) P 0.007	Entire pullback B = 324.6 (187.5, 422) F = 326 (194.7, 394.8) C = −6.9 (−22.8, 16.9) P 0.44 10 mm B = 69.4(58.8, 92.9) F = 71.7(55.7, 91.6) C = −1.9(−7.6, 6.5) P 0.73	B = 4.1 ± 0.9 F = 2.0 ± 0.5 C = −2.2 ± 0.7
Kovarnik 2012	E 42	B = 413.9 ± 239.6 F = 401.9 ± 223.1 C(mm3/mm) = −12.8 ± 38.5	B = 468.9 ± 269.8 F = 466.8 ± 258.9 C = −2.1 ± 53.3	B = 3.1 ± 1.3 F = 2 ± 0.8 %C = −28.6 ± 33.8%
	S 47	B = 420.5 ± 189.5 F = 423.3 ± 194.1 C(mm^3/mm) = 2.8 ± 3	B = 473.5 ± 205.4 F = 462.2 ± 232.9 C = −11.4 ± 70.5	B = 2.7 ± 0.8 F = 2.6 ± 0.8 %C = 1.9 ± 29.8%
Lee 2016	E 34	B = 78.3 ± 29.5 F = 74.5 ± 27.4 C = −3.7 ± 9	B = 65.9 ± 31.1 F = 65.5 ± 31.9 C = −0.4 ± 10.8	B = 111.4 ± 22 (2.9 ± 0.6) F = 68.1 ± 15.3 (1.76 ± 0.4) C = −43.4 ± 24.1 (1.1 ± 0.6)
	S 36	B = 86.5 ± 34.7 F = 86.7 ± 41.9 C = 0.3 ± 17	B = 77 ± 32.8 F = 74.6 ± 36 C = −2.4 ± 20.3	B = 119.1 ± 29.9 (3.1 ± 0.77) F = 92.2 ± 21.8 (2.39 ± 0.56) C = −26.9 ± 20.4 (0.7 ± 0.5)
Masuda 2015	E 21	B = 55.3 ± 28.4 F = 47.1 ± 24.6 C = −8.2 ± 5.33	B = 50.5 ± 31.7 F = 54.8 ± 29.7 C = 4.3 ± 6.7	B = 131.8 ± 25.6 (3.4 ± 0.66) F = 57.3 ± 20.2 (1.5 ± 0.5) %C = −55.8 ± 18.9
	S 1	B = 43.5 ± 28.5 F = 40.9 ± 24.7 C = −2.6 ± 5.34	B = 51.5 ± 31.8 F = 49.6 ± 29.8 C = −1.9 ± 9.4	B = 123 ± 27 (3.2 ± 0.7) F = 75.1 ± 21.4 (1.94 ± 0.55) %C = −36.8 ± 18.9
Nakajima 2014	E 50	B = 75.1 ± 46.6 F = 66.9 ± 45.6 C = −8.2 ± 9.5	B = 83 ± 51.3 F = 81.4 ± 55.1 C = −1.6 ± 13.9	B = 116.2 ± 24.7 mg/dL (3 ± 0.64) F = 56.8 ± 19.5 (1.47 ± 0.5) %C = −49.8% ± 19.9
	S 4	B = 76.5 ± 43.1 F = 70.3 ± 43.1 C = −6.2 ± 9.1	B = 87.5 ± 47 F = 88.9 ± 47.4 C = 1.4 ± 14.7	B = 114.3 ± 34 (2.96 ± 0.88) F = 70.3 ± 23.6 (1.8 ± 0.6) %C = −34.6 ± 27.3

(*continued on next page*)

Table S5 (*continued*)

Study ID	Study arms, n	Change in TAV, mm^3	Lumen volume, mm^3	LDL Mean ± SD
Tsujita 2015	E 100	B = 89.6(65.8–118.8)	B = 70.4 (34.5–117.1)	B = 109.8 ± 25.4 mg/dL (2.8 ± 0.66)
		F = 85.4 (65.5–110)	F = 65.8 (36.5–113.8)	F = 63.2 ± 16.3 (1.64 ± 0.42)
		C = −5.3 (−12.4 to 0.1) $P < .001$	C = −0.3 (−4.9 to 4) P 0.4	%C = −40 ± 18
	S 102	B = 84.8 (61.5–112.7)	B = 79.4 (47.5–116.6)	B = 108.3 ± 26.3 (2.8 ± 0.68)
		F = 87.2 (60.1–111.8)	F = 79.1 (47.7–115.3)	F = 73.3 ± 20.3 (1.9 ± 0.53)
		C = −1.2 (−5.7 to 3.3) $P < .1$	C = 0.8 (−5.6 to 6.9) P 0.5	%C = −29 ± 24
Wang 2015	E 50	-	-	B = 3.62 ± 1.18 F = 1.37 ± 0.83
	S 48	-	-	B = 3.48 ± 1.26 F = 1.85 ± 0.79
Ueda 2017	E 54	-	-	B = 101 ± 27 (2.62 ± 0.7) F = 75 ± 16(1.94 ± 0.4)
	S 54	-	-	B = 100 ± 27(2.59 ± 0.7) F = 61 ± 17(1.58 ± 0.4)

B, Baseline; C, Change; %Change, percent change; E, Ezetimibe treatment arm; F, Follow-up; MI, Myocardial infarction; S, Standard therapy with statin arm.

Table S6 Secondary clinical endpoints of the studies

Study ID, follow-up interval	Study arms, n	TC Mean ± SD	HDL Mean ± SD	TG Mean ± SD	Hs-CRP Mean ± SD	MACE
Hougaard 2016 (9–12 mo)	E 39	B = 5.3 ± 0.9 F = 2.9 ± 1.0 C= −2.5 ± 1.0	B = 1.1 ± 0.3 F = 1.1 ± 0.3 C = −0.06 ± 0.3	-	-	-
	P 41	B = 5.7 ± 1.0 F = 3.5 ± 0.7 C = −2.3 ± 0.7	B = 1.1 ± 0.3 F = 1.1 ± 0.3 C = −0.03 ± 0.2	-	-	-
Kovarnik 2012 (12 mo)	E 42	B = 5 ± 1.5 F = 3.5 ± 1.2 %C = −27.4 ± 24.7%	B = 1.2 ± 0.5 F = 1.2 ± 0.3 %C = 4.5 ± 40.5%	B = 1.7 ± 0.9 F = 1.2 ± 0.9 %C = −28.6 ± 33.8%	C = 0.87 ± 5.8 mg/L	-
	S 47	B = 4.6 ± 0.9 F = 4.1 ± 1.1 %C = −8±23.9%	B = 1.2 ± 0.3 F = 1.1 ± 0.3 %C = 1.3 ± 20.1%	B = 1.4 ± 0.8 F = 1.3 ± 0.8 %C = 1.9 ± 29.8%	C = 2.0 ± 7.3 mg/L	-
Lee 2016 (3 mo)	E 34	B = 190.8 ± 24.7 (4.9 ± 0.64) F = 129.7 ± 30.3 (3.4 ± 0.78) C = −61.1 ± 42.1 (1.6 ± 1.1)	B = 36 ± 8.9 (0.93 ± 0.23) F = 37.8 ± 8.7 (0.98 ± 0.22) C = 1.8 ± 7.5 (0.05 ± 0.19)	B = 120.6 ± 90.4 (1.36 ± 1.02) F = 127.6 ± 76.3 (1.44 ± 0.86) C = 7.1 ± 56.9 (0.08 ± 0.64)	B = 5.2 ± 8.3 F = 3.8 ± 7.2 C = −1.3 ± 10.9	0%
	S 36	B = 196.8 ± 38.4 (5.1 ± 0.99) F = 153.3 ± 38.5 (3.9 ± 0.99) C = −43.6 ± 37.3 (1.1 ± 0.97)	B = 39.4 ± 6.3 (1.02 ± 0.16) F = 40.7 ± 8.8 (1.05 ± 0.23) C = 1.3 ± 8.8 (0.03 ± 0.23)	B = 136.8 ± 80.9 (1.55 ± 0.9) F = 131.1 ± 64.9 (1.48 ± 0.73) C = −1.8 ± 94.4 (0.02 ± 1.1)	B = 7.6 ± 17 F = 2.1 ± 2.5 C = −5.5 ± 17.40S%	0%
Masuda 2015 (6 mo)	E 21	B = 204.4 (33.7) (5.3 ± 0.9) F = 129.5 (24.1) (3.3 ± 0.6) %C = −35.8(13.7) multiply by 0.0259 to convert chol to mmol/L, or to convert to TGA multiplied by 0.0113	B = 53.1 ± 11.8 (1.37 ± 0.3) F = 57.5 ± 15.2 (1.49 ± 0.4) %C = 8.8 ± 19.1	B = 129.7 ± 5.1 (1.47 ± 0.06) F = 84.1 ± 5.1 (0.95 ± 0.06) %C = −17.5 ± 14.5	Unit = mg/dL B = 0.092 ± 0.006 F = 0.037 ± 0.006 %C = −18.8 ± 28.7%	0% of MI, Death or Stroke. 1(3.8%) required coronary revascularization
	S 19	194 ± 35.6 (5.02 ± 0.92) F = 142.8 ± 25.5 (3.7 ± 0.66) %C = −4.6 ± 15.3 (0.12 ± 0.4)	B = 47.1 ± 12.5 (1.22 ± 0.32) F = 49.1 ± 16.1 (1.27 ± 0.42) %C = 4.3 ± 19.1	B = 144.9 ± 4.8 (1.64 ± 0.05) F = 125 ± 4.9 (1.41 ± 0.05) %C = −4.6 ± 15.3	B = 0.077 ± 0.006 F = 0.034 ± 0.006 %C = −14.4 ± 30.4%	0% of MI, Death or Stroke. 1 (4.2%) required coronary revascularization
Nakajima 2014 (6 mo)	E 50	-	B = 50.4 ± 13.5 mg/dL (1.3 ± 0.35) F = 48.6 ± 17 (1.26 ± 0.44) %C = 0.4 ± 53	B = 106.3 ± 49 (1.2 ± 0.55) F = 99.4 ± 39.8 (1.12 ± 0.45) %C = 6.4 ± 50.5	B = 1.75 ± 2.81 F = 0.05 ± 0.03 %C = −88.2 ± 17.1	0% death, MI and Stroke
	S 45	-	B = 45.5 ± 14.2 (1.18 ± 0.37) F = 48.5 ± 14.2 (1.26 ± 0.37) %C = −9.8 ± 33.5%	B = 117.5 ± 83.5 (1.32 ± 0.94) F = 126.4 ± 70.2 (1.43 ± 0.79) %C = 22.8 ± 50.9	B = 0.81 ± 1.9 F = 0.12 ± 0.14 %C = −39.2 ± 14.2	0% death, MI and stroke

(*continued on next page*)

Table S6 (*continued*)

Study ID, follow-up interval	Study arms, n	TC Mean ± SD	HDL Mean ± SD	TG Mean ± SD	Hs-CRP Mean ± SD	MACE
Tsujita 2015 (9–12 mo)	E 100	B = 177.3 ± 32.4 mg/dL (4.6 ± 0.84) F = 129.4 ± 22 (3.3 ± 0.57) %C = −25 ± 17	B = 41.1 ± 9.5 (1.06 ± 0.25) F = 45.6 ± 11.9 (1.18 ± 0.31) %C = 14 ± 26%	B = 114 (81 to158) F = 92 (76–120) %C = −14(−33 to 18)	B = 3 (1–14.9) F = 0.4 (0.2–1.3) %C = −89(−97 to −59)	24 of 121.[20] Note. 121 is the no. from Per-Protocol Analysis
	S 102	B = 172.7 ± 32.6 (4.5 ± 0.84) F = 138.7 ± 26.2 (3.6 ± 0.68) %C = −18 ± 18	B = 40 ± 10.3 (1.04 ± 0.27) F = 43.3 ± 11.5 (1.12 ± 0.3) %C = 11 ± 25	B = 116 (92–159) F = 111 (87–139) %C = −9 (−33 to 25)	B = 3.7 (1.2–8.1) F = 0.3 (0.2–0.8) %C = −86(−95 to −70)	24 of 122. Note. Same as above
Wang 2015 (12 mo)	E 50	B = 5.65 ± 2.47 F = 3.21 ± 0.82 (12 mo)	B = 1.13 ± 0.21 F = 1.26 ± 0.41	B = 1.97 ± 0.67 F = 1.19 ± 0.32	B = 9.35 ± 3.21 F = 2.04 ± 1.71	MI = 0% Recurrent MI = 0% Unstable angina = 2 (4%) Cardiac death = 0% Stroke = 0%
	S 48	B = 5.58 ± 2.58 F = 4.02 ± 0.91 (12 mo)	B = 1.13 ± 0.22 F = 1.3 ± 0.49	B = 1.9 ± 0.65 F = 1.76 ± 0.38	B = 9.43 ± 3.11 F = 3.17 ± 1.49	MI = 1 (2.1%) Recurrent MI = 0% Unstable angina = 5 (10.4%) Cardiac death = 0% Stroke = 0%
Ueda 2017 (9 mo)	E 54	B = 168 ± 36(4.3 ± 0.93) F = 126 ± 25(3.3 ± 0.65)	B = 47 ± 19(1.22 ± 0.49) F = 44 ± 12(1.14 ± 0.31)	B = 114 ± 58(1.3 ± 0.6) F = 95 ± 46(1.1 ± 0.5)	Unit = ng/dL B = 7653 ± 11,370 F = 1699 ± 2830	-
	S 54	B = 162 ± 33 (4.2 ± 0.8) F = 140 ± 21(3.6 ± 0.5)	B = 45 ± 9(1.16 ± 0.23) F = 45 ± 11(1.16 ± 0.28)	B = 113 ± 52 (1.3 ± 0.6) F = 109 ± 54 (1.2 ± 0.6)	Unit = ng/dL B = 5510 ± 13,018 F = 988 ± 1521	-

B, baseline; C, change; %Change, percent change; E, ezetimibe treatment arm; F, follow-up; MI, myocardial infarction; S, standard therapy with statin arm.

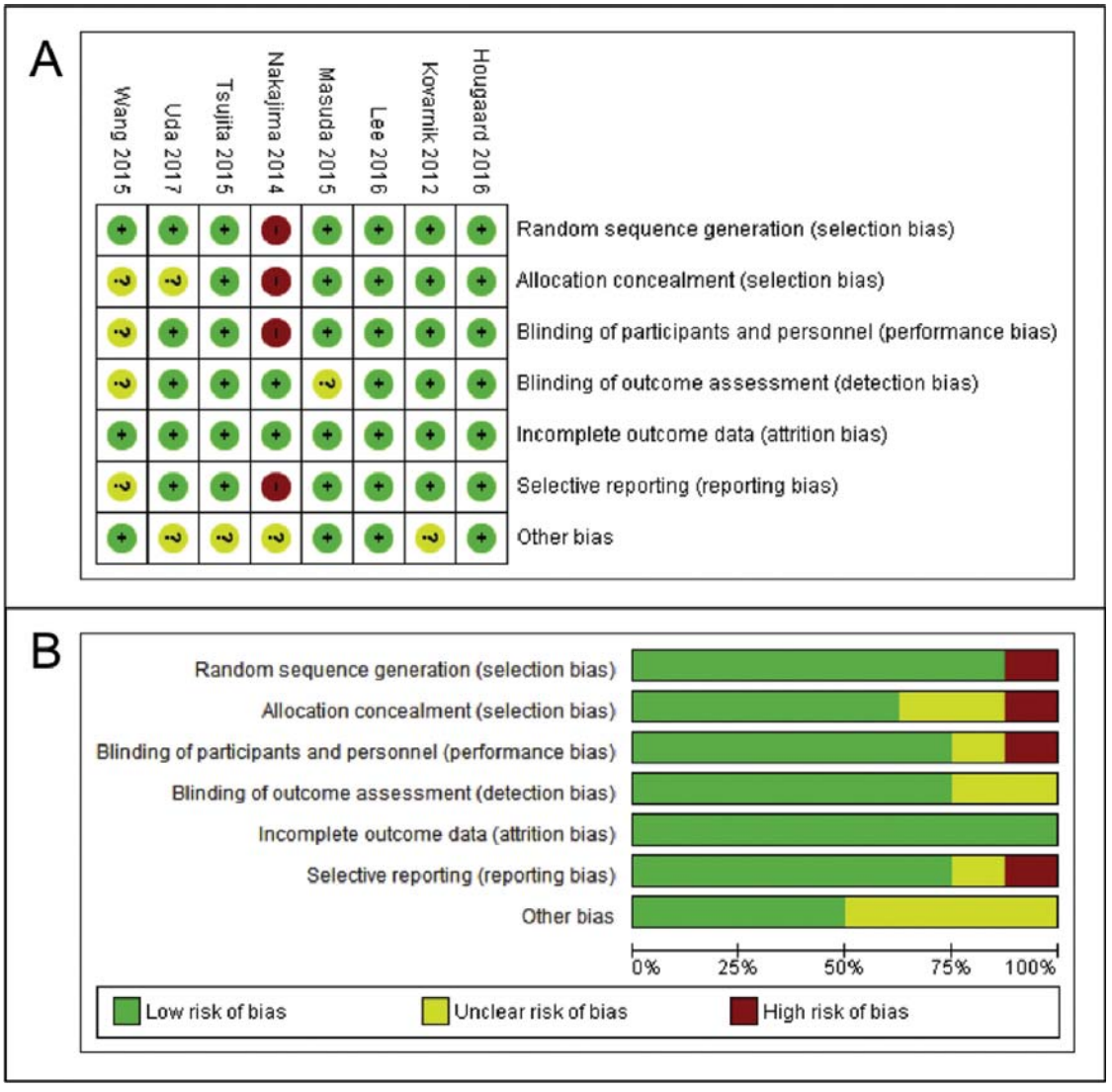


Figure S1 (A) Individual bias assessment of included studies. (B) Summary bias assessment of included studies.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Impact of Ezetimibe + Statin therapy on coronary plaque regression (IVUS)	Statin alone therapy	Relative (95% CI)	Absolute (95% CI)		
Coronary plaque regression												
8	randomised trials	not serious	not serious	not serious	not serious	strong association	340	341	-	MD **4.34 lower** (6.4 lower to 2.29 lower)	⨁⨁⨁⨁ HIGH	CRITICAL
Change in LDL by percentage												
4	randomised trials	not serious	not serious	not serious	not serious	strong association	213	213	-	MD **17.43 lower** (25.08 lower to 9.78 lower)	⨁⨁⨁⨁ HIGH	CRITICAL
Change in total cholesterol												
3	randomised trials	not serious	not serious	not serious	not serious	strong association	163	168	-	MD **18.85 lower** (34.22 lower to 3.48 lower)	⨁⨁⨁⨁ HIGH	CRITICAL
Change in HDL												
4	randomised trials	not serious	not serious	not serious	not serious	strong association	213	213	-	MD **3.97 higher** (1.3 lower to 9.24 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT
Change in triglyceride level												
4	randomised trials	not serious	not serious	not serious	not serious	none	213	213	-	MD **1.32 lower** (19.75 lower to 17.12 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT

CI: Confidence interval; **MD:** Mean difference

Figure S2 Grading of Recommendations, Assessment, Development and Evaluation assessment of primary and secondary outcomes.

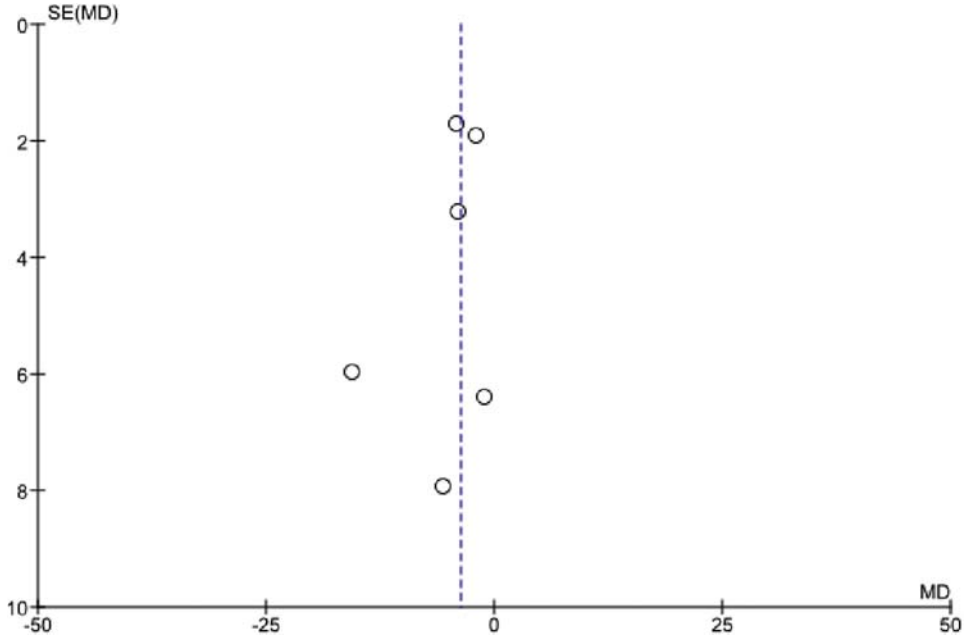


Figure S3 Funnel Plot of comparison studies for primary end of delta TAV using IVUS.

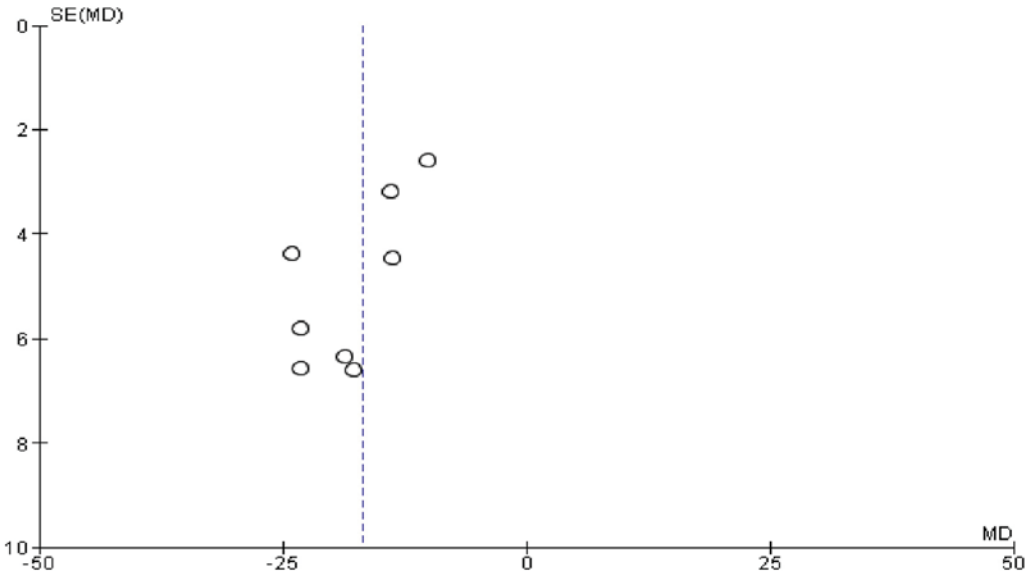


Figure S4 Funnel plot of comparison: 2 Impact of Ezetimibe on plasma LDL-C level [mg/dL], outcome: 2.1 Impact of Ezetimibe on plasma LDL-C level.

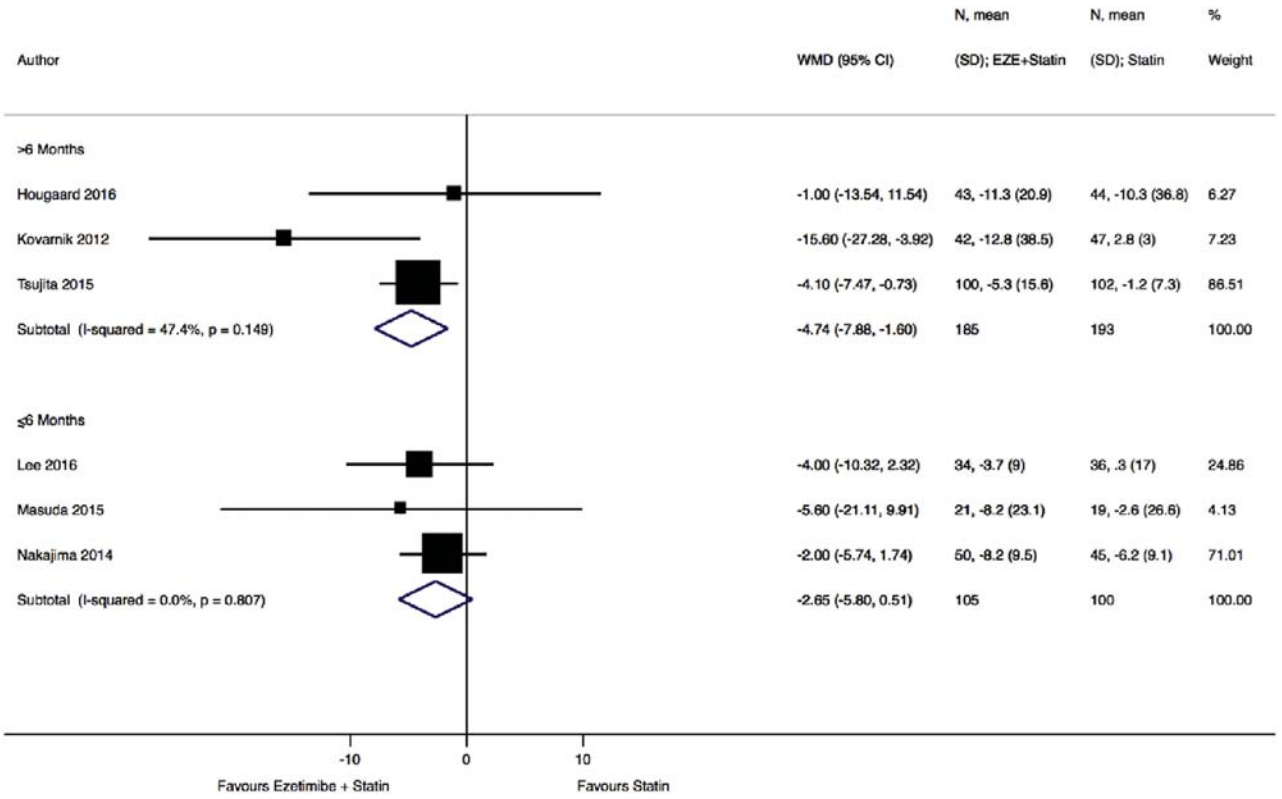


Figure S5 Forest Plot of sensitivity analysis for length of follow-up (>6 mo vs ≤ 6 mo) in relation with primary endpoint of change in TAV using IVUS.

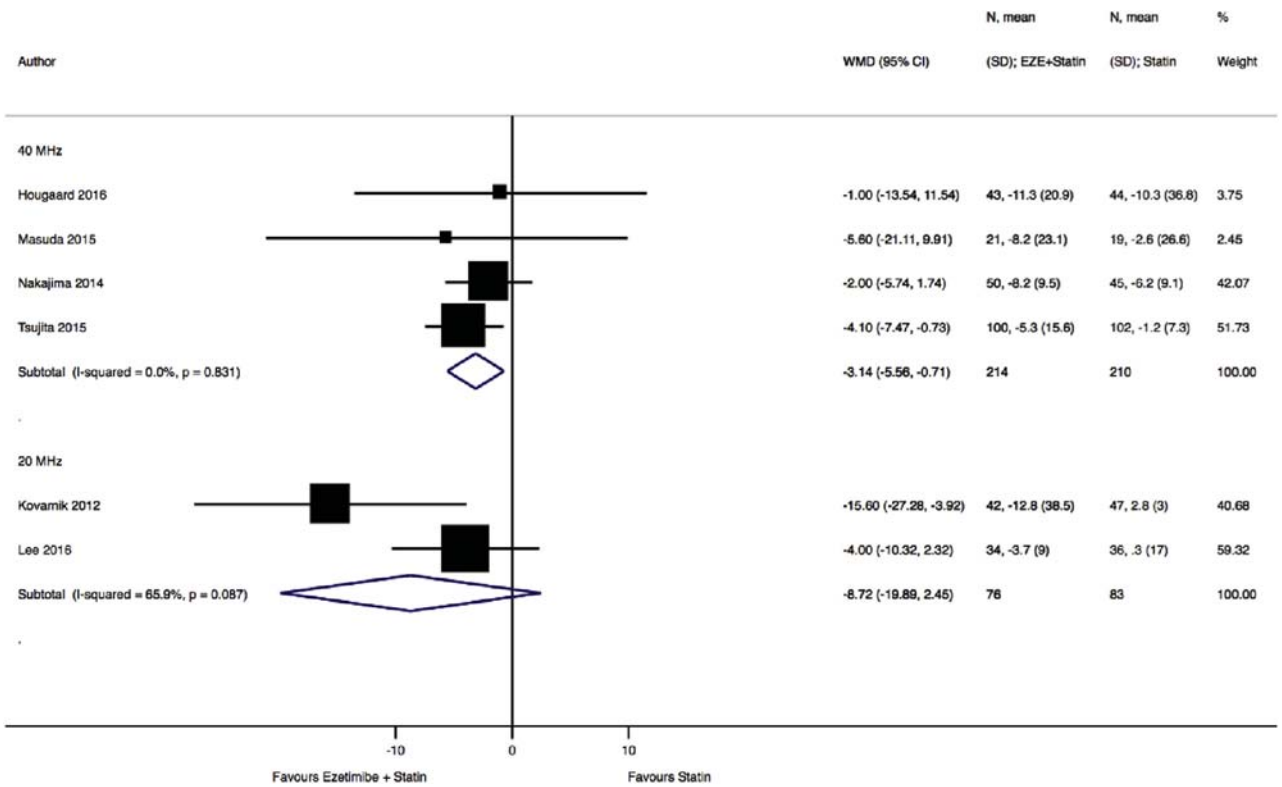


Figure S6 Forest Plot of sensitivity analysis for IVUS catheter resolution (40 MHz vs 20 MHz) in relation with primary endpoint of change in TAV using IVUS.

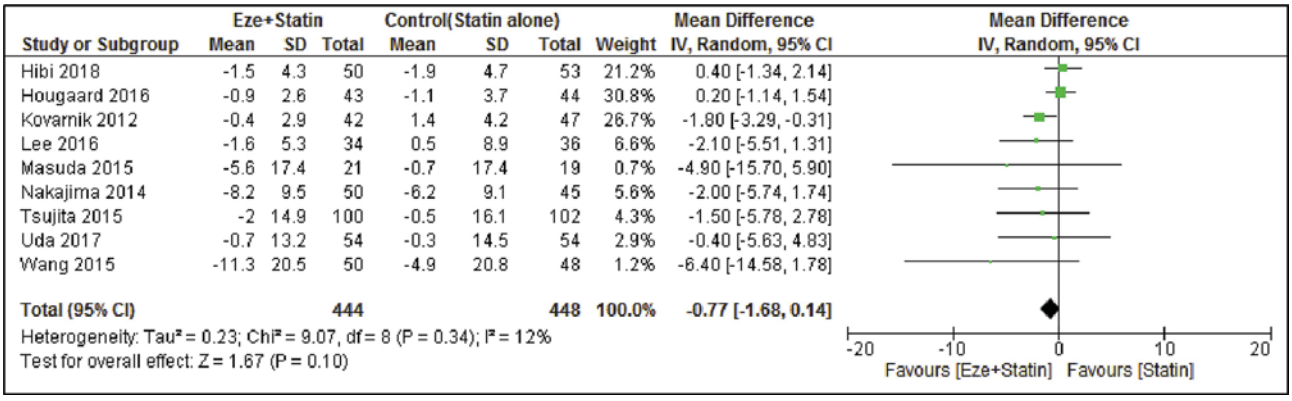


Figure S7 Forest plot of change in PAV (%) assessed via IVUS. IVUS, intravascular imaging ultrasound; PAV, percent atheroma volume.

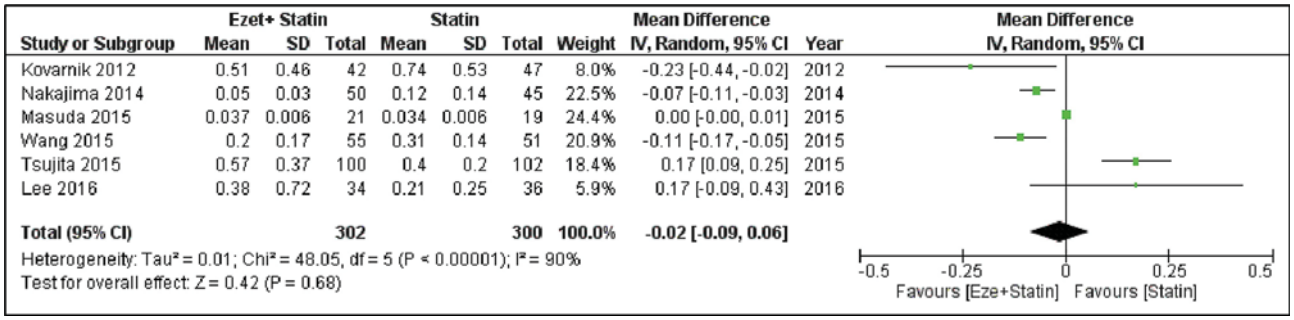


Figure S8 Forest plot of change in LV (mm^3) assessed via IVUS. IVUS, intravascular imaging ultrasound; LV, luminal volume.

Chapter 3

Prevalence, Prognosis, Knowledge and Awareness of Familial Hypercholesterolemia in a Coronary Care Unit

CHAPTER 3

Preface

Current evidence demonstrates heterozygous FH is a relatively common autosomal monogenic disorder of lipid metabolism associated with a significantly increased lifetime risk of premature coronary artery disease. On the other hand, recent data has shown the prevalence of FH is significantly underestimated and FH patients are ndertreated. We hypothesized that the coronary care unit (CCU) will be a unique location for systematic screening of FH among patients with premature acute coronary syndrome. Screening for FH patients in CCU requires a certain level of knowledge and clinical vigilance, hence, we first conducted a multicenter survey to assess awareness and knowledge of FH amongst health providers managing acute coronary syndrome. We performed a retrospective study to outline frequency of FH, and the impact of FH on the prognosis of acute coronary syndrome compared to non-FH patients. In addition to CCU, we also evaluated the role of the tertiary hospital laboratory as another avenue for opportunistic screening for FH in hospital. This chapter contains three original research papers.

Chapter 3 A

Awareness of Familial Hypercholesterolemia among Healthcare Providers Involved in the Management of Acute Coronary Syndrome in Victoria, Australia.

CJC Open 1 (2019) 168–172

Original Article

Awareness of Familial Hypercholesterolemia Among Healthcare Providers Involved in the Management of Acute Coronary Syndrome in Victoria, Australia

Sam Mirzaee, MD, MPH,[a] Hashrul N. Rashid, MBBS(Hons),[a] Odgerel Tumur, MBBS,[b] Jason Nogic, MBBS,[a,c] Kunal Verma, MBBS,[d] James D. Cameron, MBBS, MD, BE(Elec), MEngSc,[a] Stephen J. Nicholls, MBBS, PhD,[a] and Arthur Nasis, MBBS, MD, PhD[a]

[a] *Monash Cardiovascular Research Centre MonashHeart, Monash Health, Monash University, Melbourne, Australia*

[b] *The Royal Melbourne Hospital, Cardiology Department, Melbourne, Australia*

[c] *Box Hill Hospital, Eastern Health, Cardiology Department, Melbourne, Australia*

[d] *Western Hospital, Western Health, Cardiology Department, Melbourne, Australia*

ABSTRACT

Background: Familial hypercholesterolemia (FH) is a common underdiagnosed autosomal dominant lipid disorder carrying a significant risk of premature coronary artery disease. The aim of this study was to evaluate the awareness and knowledge of heterozygous FH of healthcare providers in coronary care units (CCUs).

Methods: Medical staff working in CCUs in 4 sizable metropolitan health networks in Melbourne, Australia, were requested to complete a structured anonymised questionnaire with regard to FH. The results were tabulated and analysed with the Statistical Package for the Social Sciences version 23 (IBM, New York, NY).

Results: A total of 121 participants (67% response rate) completed the survey. Some 76% claimed to be at least modestly familiar with

RÉSUMÉ

Contexte : L'hypercholestérolémie familiale (HF) est un trouble lipidique autosomique dominant courant et sous-diagnostiqué, associé à un risque important de coronaropathie prématurée. Le but de cette étude consistait à évaluer la sensibilisation et les connaissances à l'égard de l'HF hétérozygote parmi les professionnels de la santé œuvrant en unité de soins coronariens (USC).

Méthodologie : Les membres du personnel médical des USC de quatre réseaux de santé métropolitains relativement importants de Melbourne, en Australie, ont été invités à remplir un questionnaire anonyme structuré sur l'HF. Les résultats ont été mis sous forme de tableaux et analysés à l'aide de la trousse logicielle SPSS (*Statistical Package for the Social Sciences*, IBM, New York, NY), version 23.

Epidemiological evidence demonstrates familial hypercholesterolemia (FH) as a common genetic cause of long-standing elevated plasma level of low-density lipoprotein cholesterol (LDL-C), causing accelerated development of atherosclerotic cardiovascular disease by at least 1 to 2 decades.[1] Globally, it is estimated that 20 million people are affected by FH, of whom approximately 45,000 are living in Australia, with the majority of them currently unidentified and inadequately treated.[1,2]

The actual prevalence of heterozygous FH in the general population has been reported as up to 1:200 to 250, which is substantially higher than was formerly estimated at approximately 1:500.[1] Although the diagnostic criteria for FH remain debatable, the Dutch Lipid Clinic Network criteria[1] is the currently preferred method for detecting index cases in Australia. A recent Australian study reported a high prevalence of phenotypical FH (~14%) among patients in a coronary care unit (CCU) who were identified with premature coronary artery disease.[3] The precise frequency of FH in patients with acute coronary syndrome (ACS) is unclear and has been broadly reported as varying from 1.60% to 15.40% among diverse ethnic populations.[3-5]

A large body of epidemiological evidence shows that the majority of patients with FH have had inadequate

Received for publication March 9, 2019. Accepted May 7, 2019.

Ethics Statement: This research has adhered to the national ethical guideline.

Corresponding author: Dr Sam Mirzaee, 246 Clayton Road, Clayton, Victoria 3168, Australia. Tel.: +61 3 9594 6666; fax: +61 3 9594 6239.

E-mail: Sam.Mirzaee@MonashHealth.org

See page 171 for disclosure information.

https://doi.org/10.1016/j.cjco.2019.05.001

FH, and more than half of them adequately described FH; however, only 16% and 43%, respectively, were aware of the prevalence of FH and existence of lipid guidelines. In regard to epidemiological knowledge and update in the management of FH in CCUs, knowledge was suboptimal. In regard to FH care, General Practitioners were rated by 72% of participants as the first most efficient healthcare provider in the management of FH, and cardiologists were rated by 54% of participants as the second most efficient healthcare provider in the management of FH. Some 36% of respondents advocated a form of alert system in laboratory reports to facilitate the diagnosis of FH.

Conclusions: This survey identified substantial gaps in the knowledge and awareness of FH among healthcare providers involved in the management of acute coronary syndrome. Focused education and clinical training are warranted to raise awareness of FH among healthcare providers working in CCUs.

Résultats : Au total, 121 personnes (taux de réponse de 67 %) ont participé à l'enquête. Environ 76 % des répondants ont indiqué posséder à tout le moins quelques connaissances sur l'HF, tandis que plus de la moitié d'entre eux en ont donné une définition adéquate; en revanche, seuls 16 et 43 %, respectivement, connaissaient la prévalence de l'HF et l'existence de lignes directrices sur les lipides. Par rapport aux connaissances épidémiologiques et à l'actualisation des stratégies de prise en charge de l'HF en USC, les connaissances étaient sous-optimales. Soixante-douze pour cent des répondants ont jugé que le médecin généraliste était le professionnel de la santé le plus à même de soigner et de prendre en charge l'HF; le cardiologue a été mentionné en seconde position par 54 % des répondants. Quelque 36 % des répondants ont préconisé la mise en place d'un système d'alerte, dans les rapports de laboratoire, pour faciliter le diagnostic d'HF.

Conclusions : Cette enquête a mis en évidence des lacunes considérables dans la sensibilisation et les connaissances à l'égard de l'HF parmi les professionnels de la santé intervenant dans la prise en charge du syndrome coronarien aigu. Un enseignement et une formation clinique ciblés s'imposent pour accroître la sensibilisation à l'égard de l'HF parmi les professionnels de la santé qui travaillent en USC.

LDL-C—lowering management.[6] Even with the widespread use of statins, patients with FH and ACS remain at higher risk of recurrent events within the first 12 months after discharge from cardiac care.[7] This perhaps reflects both the refractory nature of elevated LDL-C in FH and a possible suboptimal lipid-lowering therapy. To assist treating physicians and to raise awareness, several guidelines and models of care for FH have been provided by Cardiovascular Societies.[8,9] There has also been recent advancement in the range of pharmacological management available with associated reduction of cardiovascular events in patients with FH after the introduction of proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors.[10]

Suboptimal awareness of the importance of FH among community healthcare providers in Australia has been demonstrated.[11] The outcome of this Australian survey was consistent with other international studies demonstrating a deficit in the knowledge and awareness of FH among primary care physicians.[12-14] Analogous findings were reported in a cross-sectional multicenter study among tertiary hospital physicians in the Persian Gulf region.[15] A report from the United States indicated a fundamental FH education gap in the curriculum within both medical and pharmacy training.[16]

When sought for, FH is relatively common in CCUs,[3] yet there has not been an extensive survey to evaluate the knowledge and awareness of healthcare providers working in the CCU. Therefore, we designed a cross-sectional study to assess the awareness of heterozygous FH in this potentially influential group of care providers. We hypothesized that high FH awareness in CCUs would allow the diagnosis of index cases and, subsequently, appropriate treatment for patients with FH and their first-degree relatives. Improved identification would allow provision of an adequate lipid-lowering regimen for primary and secondary prevention, patient education, cascade screening, and optimization of the overall management of heterozygous FH in the community.

Methods

Subjects

Cardiologists, cardiology trainees, and cardiac nurses working in 4 large tertiary health networks in Melbourne, Australia, were approached between January and June 2018. We selected our population study by a multistage sampling method. Multistage sampling was obtained by using 2 stages. The first stage involved a random selection of 4 different health networks based on the distribution of demographics. In the second stage, a random sample of the nested population within the selected district was obtained. Multistage sampling was particularly used in preference to simple random sampling because the population was geographically diverse in Victoria. Subsequently, systemic random sampling was applied by including those who met the following criteria: (1) consultant cardiologists, cardiology fellows, cardiology registrars, and cardiac care nurses with at least 2 years clinical experience since graduation; (2) minimum half of practice time in a clinical setting; (3) attend to $\geq$ 50 patients per month. The eligible clinicians were asked to complete an anonymous print-based survey without time compensation. The participants received no financial motivation, the survey was entirely voluntary, and announcements were made by a dedicated site researcher in the routine educational meeting by inviting the staff to participate in the study if they were interested.

Questionnaire

The measurement tool was primarily adapted from a study involving General Practitioners (GPs) in primary care by Bell et al.[11] in 2014, in conjunction with experts' recommendations on FH and national guidelines.[2,3] The study questionnaire has 23 comprehensive questions, a mix of opinion questions, and quiz (1 correct answer) questions on the important aspects of FH: awareness of the condition, clinical manifestation, epidemiology, genetics, risk of atherosclerotic cardiovascular disease, and choices and awareness of explicit

lipid services (Supplemental Appendix S1). Despite the lack of a standard method to assess FH awareness, we designed our comprehensive questions in this tool to yield a minimum variability in answers from the healthcare providers in CCUs. Printed survey forms were distributed among participants, and adequate time was provided for completion. They were not allowed to discuss the questions with each other nor was searching electronically permitted.

Analysis

Analysis was performed using Microsoft Excel 2013 (Microsoft Corp, Redmond, WA) and the Statistical Package for the Social Sciences version 23.0.0 (IBM, New York, NY). Descriptive statistics were presented as percentages for the discrete variables and as median for the continuous variables. Results were rounded to the nearest tenth. The chi-square test was used to assess whether the years of clinical experience as a categorical variable ($\leq$ 10 years) was a determinant of difference in FH knowledge and practice.

Ethics

The proposal was approved by the institutions' Human Research Ethics Committees (September 2017), in accordance with the National Health and Medical Research Council. Informed consent was not required.

Results

Participants' details

A total of 180 potentially eligible staff were randomly approached; 28 did not meet the inclusion criteria (mostly because of insufficient practice time in the CCU), 13 did not return the survey, and 18 did not entirely complete the questionnaire. A total of 121 participants (67% response rate) completed the survey; 37% were cardiologists (n = 45), 23% were cardiology fellows/registrars (n = 28), and 40% were cardiac care nurses (n = 48). The majority of participants had more than 5 years of clinical experience working in CCUs (62%) and described their workload as moderate or high, treating at least 50 patients per month (75%) (Table 1).

Table 1. Basic characteristics of participants

Characteristics	N (%)
Respondents	121 (67)
Clinical background	
Consultant Cardiologist	45 (37)
Cardiology Fellow	12 (10)
Registrar in Training	16 (13)
Cardiac Nurse	48 (40)
Clinical work experience	
> 5 y	75 (62)
< 5 y	46 (38)
Clinical workload	
Above moderate (≥ 50 patients monthly)	91 (75)
Below moderate (30-50 patients monthly)	30 (25)
Self-concept of familiarity with FH	
Average and above	92 (76)
Below average	29 (24)

FH, familial hypercholesterolemia.

Awareness and knowledge of FH

In regard to awareness of FH, 76% considered their familiarity with FH as average, 43% were aware of current lipid guidelines, 56% were aware of PCSK9 inhibitors as a modality of treatment for FH, and only 36% were aware of available clinical lipid services (Supplemental Appendix S1).

In regard to knowledge of FH, 63% were able to describe and characterize FH correctly, 68% recognized the lipid profile consistent with FH, and 56% accurately selected the requirement of genetic testing for an accurate diagnosis. Regarding identifying the prevalence of FH, only 16% and 19% were able to correctly specify the prevalence of this condition in the general population and coronary care settings, respectively. Concerning cardiovascular risk in FH, 18% recognized the age definition for premature cardiovascular disease, and 48% accurately identified the cardiovascular disease risk in untreated FH and rate of coronary events recurrence in patients with FH (Table 2). The length of clinical experience working in CCUs ($\leq$ 10 years) was not statistically associated with FH knowledge ($P = 0.76$).

Screening FH

With regard to their opinion on which healthcare provider was best placed to detect FH, 72% considered GPs as the most efficient healthcare provider for the timely management of FH, with 54% identifying cardiologists as the second most efficient healthcare provider. Some 36% thought a laboratory report alert system would be useful assistance in the early detection of FH. With regard to previous experience in managing FH, 44% had managed patients with FH, 8% would routinely screen the close relatives of patients with FH, and 45% would perform phenotypical screening for FH in patients with premature cardiovascular disease. The majority believed the age group of 13 to 18 years as the most appropriate for screening young individuals in a family with premature ACS (42.9%), and only 3.3% nominated below the age of 6 years as an appropriate time for screening.

Discussion

To the best of our knowledge, this is the first specific formal survey of FH awareness in staff in an Australian coronary care setting. Although a majority of staff claimed knowledge of FH as adequate or above, the results suggest that there is a notable scope for improvement in the area of FH recognition and management. The survey results are in line with current literature indicating shortcomings in the knowledge of FH, including an unawareness of the prevalence, the early onset of coronary artery disease, and the fact that FH is the most common and underdiagnosed hereditary lipid disorder.[12-14]

Fifty-six percent of CCU staff stated they had not had former experience in managing patients with FH, potentially indicating that the importance of FH has often been underestimated in the coronary care setting. GPs and cardiologists were nominated by the participants as the most appropriate healthcare providers in the diagnosis and management of FH. A majority of participants (> 60%) were unaware of specialist

Table 2. Summary of coronary care staff replies to questions about FH

Awareness and knowledge	Proportion
Considered familiar with FH ranked as average	76%
Aware of guidelines	43%
Awareness of any specialised clinical lipid services to whom you can refer patient	36%
Familiarity with PCSK9 inhibitors for treatment of FH ranked as average or above	56%
Properly described FH	63%
Properly recognised the lipid profile	68%
Properly identified the prevalence of FH in the community	16%
Properly identified the prevalence of FH in the CCU	19%
Properly identified the rate of coronary events recurrence in patients with FH	48%
Properly selected the age definition for premature CVD	18%
Properly selected the requirement of genetic testing for accurate diagnosis of FH	56%
Opinion on healthcare provider to detect early FH	
Selected GPs as the most effective healthcare provider for the early diagnosis of FH	72%
Selected cardiologist as the second most effective healthcare provider for the early diagnosis of FH	54%
Selected laboratory report alerting system as a useful assistant in early detection of FH	36%
Practice and previous experience on management of patients with FH	
Phenotypical screening for FH in premature CVD	45%
Screening family members for FH	8%
Previous experience in care of patients with FH	44%
Age 0-6 y was selected as the appropriate age for screening young individuals for FH in a family with premature CAD	3%

CAD, coronary artery disease; CCU, coronary care unit; CVD, cardiovascular disease; FH, familial hypercholesterolemia; GP, General Practitioner; PCSK9, proprotein convertase subtilisin/kexin 9.

lipid clinics, which are considered essential for the adequate treatment of individuals identified with FH.[2] More than one-third of participants were not familiar with PCSK9 inhibitors as a novel recently available LDL-C modulator. PCSK9 inhibitors are available in well-developed healthcare systems, including in Australia, and they are an effective LDL-C—lowering therapy to reduce the risk of cardiovascular events.[17] Although there is no consensus with regard to the age of testing and commencement of statin therapy in children with a first-degree relative with FH, screening from age 5 years or even younger is recommended if homozygous FH is clinically suspected.[18]

Recognition of the importance of familial screening in FH, in particular of offspring, was markedly suboptimal in this survey. Poor documentation of family history with inadequate treatment plans in the medical records of both primary and tertiary practices may perpetuate this deficiency.[19,20] This is important because, in contrast to the index case detection, the diagnosis of FH in immediate relatives by cascade screening has been shown to be effective and inexpensive.[21] Although the traditional diagnosis of FH is clinical, genetic testing to increase the accuracy of early diagnosis in addition to facilitating further cascade screening in their relatives is recommended.[22]

Because FH is a relatively common, but treatable lipid disorder, the World Health Organization criteria for disease screening in FH is applicable.[23] Cascade screening paves the way for subsequent diagnosis and treatment of FH in the first-degree relatives of index patients with early-onset coronary artery disease admitted to the CCU as part of a primary prevention strategy. Also, this will draw attention to the management of the index cases with FH who have already developed ACS for optimization of LDL-C—lowering therapy to improve secondary prevention. The strength of this study is that for the first time in Australia, data are exclusively addressing the awareness of FH among healthcare providers working in CCUs.

Study limitations

This study had a modest sample size; however, the response rate is noticeably high. To a certain extent, the selection of 4 health institutions was related to accessibility and the print-based design of the survey. It is possible that the knowledge gap is greater in subjects who did not complete the survey. We did not inquire about knowledge of other aspects, such as lipoprotein(a), which has a higher prevalence in FH, screening for elevated lipoprotein(a), or potential use of lipid apheresis for treatment. Finally, this survey was performed in only 4 health networks, located diversely in Melbourne metropolitan regions that may not reflect the overall knowledge of FH in Australia.

Conclusion

This qualitative study draws attention to a suboptimal FH awareness and proficiency of hospital-based healthcare providers involved in the management of ACS. Education and a holistic approach toward patients with FH through an integrated model of care, led by a dual trained cardiologist and lipid specialist, may enhance the current standard of care for patients with FH in CCUs across Australia.

Acknowledgements

The authors thank Jonathan Yan Ho Lee and Jacquelyn Teo Yi Xin for assistance in data acquisition.

Disclosures

Dr Mirzaee has received consultancy fees from Amgen. Dr Nicholls reports receiving research support from AstraZeneca, Cerenis, Amgen, Novartis, Eli Lilly, Anthera, LipoScience, Roche, Sanofi-Regeneron, and Resverlogix, and receiving honoraria from or serving as a consultant for Amgen, AstraZeneca, Roche, Esperion, Eli Lilly, Abbott, Pfizer, Merck, Takeda, LipoScience, Omthera, Novo-Nordisk, Sanofi-Regeneron, Atheronova, Anthera, CSL Behring, and Boehringer Ingelheim. The other authors have no conflicts of interest to disclose.

References

1. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J 2013;34:3478-90a.

2. Watts GF, Sullivan DR, Poplawski N, et al. Familial hypercholesterolaemia: a model of care for Australasia. Atheroscler Suppl 2011;12:221-63.

3. Pang J, Poulter EB, Bell DA, et al. Frequency of familial hypercholesterolemia in patients with early-onset coronary artery disease admitted to a coronary care unit. J Clin Lipidol 2015;9:703-8.

4. De Backer G, Besseling J, Chapman J, et al. Prevalence and management of familial hypercholesterolaemia in coronary patients: an analysis of EUROASPIRE IV, a study of the European Society of Cardiology. Atherosclerosis 2015;241:169-75.

5. Nanchen D, Gencer B, Auer R, et al. Prevalence and management of familial hypercholesterolaemia in patients with acute coronary syndromes. Eur Heart J 2015;36:2438-45.

6. deGoma EM, Ahmad ZS, O'Brien EC, et al. Treatment gaps in adults with heterozygous familial hypercholesterolemia in the United States data from the CASCADE-FH Registry. Circ Cardiovasc Genet 2016;9:240-9.

7. Nanchen D, Gencer B, Muller O, et al. Prognosis of patients with familial hypercholesterolemia after acute coronary syndromes. Circulation 2016;134:698-709.

8. Watts GF, Sullivan DR, van Bockxmeer FM, et al. A model of care for familial hypercholesterolaemia: key role for clinical biochemistry. Clin Biochem Rev 2012;33:25-31.

9. Brunham LR, Ruel I, Aljenedil S, et al. Canadian Cardiovascular Society Position Statement on Familial Hypercholesterolemia: Update 2018. Can J Cardiol 2018;34:1553-63.

10. Bell DA, Watts GF. Progress in the care of familial hypercholesterolaemia: 2016. Med J Aust 2016;205:232-6.

11. Bell DA, Garton-Smith J, Vickery A, et al. Familial hypercholesterolaemia in primary care: knowledge and practices among general practitioners in Western Australia. Heart Lung Circ 2014;23:309-13.

12. Rangarajan N, Balasubramanian S, Pang J, Watts GF. Knowledge and awareness of familial hypercholesterolaemia among registered medical practitioners in Tamil Nadu: are they suboptimal? J Clin Diagn Res 2016;10:OC52-6.

13. Pang J, Sullivan DR, Harada-Shiba M, et al. Significant gaps in awareness of familial hypercholesterolemia among physicians in selected Asia-Pacific countries: a pilot study. J Clin Lipidol 2015;9:42-8.

14. Pang J, Hu M, Lin J, et al. An enquiry based on a standardised questionnaire into knowledge, awareness and preferences concerning the care of familial hypercholesterolaemia among primary care physicians in the Asia-Pacific region: the "Ten Countries Study". BMJ Open 2017;7.

15. Batais MA, Almigbal TH, Bin Abdulhak AA, et al. Assessment of physicians' awareness and knowledge of familial hypercholesterolemia in Saudi Arabia: is there a gap? PLoS One 2017;12:e0183494.

16. Withycombe B, Winden JC, Hassanyn R, Duell PB, Ito MK. The extent of familial hypercholesterolemia instruction in US schools and colleges of medicine, pharmacy, and osteopathic medicine. J Clin Lipidol 2015;9:281-8.

17. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 2017;376:1713-22.

18. Wiegman A, Gidding SS, Watts GF, et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J 2015;36:2425-37.

19. Qureshi N, Armstrong S, Dhiman P, et al. Effect of adding systematic family history enquiry to cardiovascular disease risk assessment in primary care: a matched-pair, cluster randomized trial. Ann Intern Med 2012;156:253-62.

20. Bates TR, Poulter EB, van Bockxmeer FM, Watts G. Family history: the neglected risk factor in disease prevention. Med J Aust 2010;193:429-30.

21. Ned RM, Sijbrands EJ. Cascade screening for familial hypercholesterolemia (FH). PLoS Curr 2011;3:RRN1238.

22. Amor-Salamanca A, Castillo S, Gonzalez-Vioque E, et al. Genetically confirmed familial hypercholesterolemia in patients with acute coronary syndrome. J Am Coll Cardiol 2017;70:1732-40.

23. Schofield J, Kwok S, France M, et al. Knowledge gaps in the management of familial hypercholesterolaemia. A UK based survey. Atherosclerosis 2016;252:161-5.

Supplementary Material

To access the supplementary material accompanying this article, visit *CJC Open* at https://www.cjcopen.ca/ and at https://doi.org/10.1016/j.cjco.2019.05.001.

Chapter 3 B

Familial Hypercholesterolemia and Incidence of Fatal Ventricular Arrhythmias Complicating Premature Acute Coronary Syndrome

Familial Hypercholesterolemia and Incidence of Fatal Ventricular Arrhythmias Complicating Premature Acute Coronary Syndrome

Sam Mirzaee MD, MPH [1], Andrew Lin MBBS, BMed Sci [1], James D Cameron MBBS MD BE MEngSc[1] (Elec)

[1] *Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University Melbourne, Australia.*

Corresponding Author:

Dr Sam Mirzaee

Monash Cardiovascular Research Centre, MonashHeart, Monash Health

246 Clayton Road, Clayton, Vic 3150 Australia

Telephone: +61 9594 6666

Email: sammirzaee@hotmail.com

Heterozygous Familial hypercholesterolemia (FH) is a common inherited lipid disease characterized by high plasma levels of low-density lipoprotein cholesterol (LDL-C), increased risk of premature coronary artery disease (CAD) and further associated complications. [1] Primary ventricular fibrillation (VF) is the leading cause of mortality in acute myocardial infarction (AMI). [162] However, there are limited prognosis data in patients with FH on the background of AMI. Dekker at al. reported a lower rate of primary VF associated with AMI in a subgroup with significant hypercholesterolemia, suggesting a potential multifactorial mechanism for primary VF. [162] Likewise, a recent murine study demonstrated a negative association between dyscholesterolemia and occurrence of re-entry arrhythmia in AMI due to the potential modification of the lipid content of myocytes and further modulation of voltage-gated ion channel function resulting in prolongation of the action potential and QT interval duration of the ECG. [163] The GISSI-Prevenzione trial also demonstrated an inverse relationship between consumption of polyunsaturated fatty acids and fatal arrhythmias via remodeling of voltage-dependent Na^+ and L-type Ca^{2+} channels in the cell membrane. [164]

Accordingly, we hypothesised that patients with FH might have similar electrophysiological changes due to high cumulative LDL-C levels resulting in moderated incidence of fatal arrhythmias following AMI. We included 231 patients with early onset AMI (age <55 years for males and <60 years for females) out of 997 total admissions to the coronary care unit in a large tertiary centre between January-December 2015. Using the modified Dutch Lipid Clinic Network (DLCN) Criteria [5] : history of CAD (2 points), or stroke (1 point); premature family history of CAD (1 point); and plasma LDL-C >8.5 mmol/L (8 points), 6.5–8.4 mmol/L (5 points), 5–6.4 mmol/L (3 points), 4–4.9 mmol/L (1 point). FH likelihood was calculated by summation of the DLCN criteria: unlikely, score <3; possible, score 3–5; and probable/definite FH score ≥6. We defined phenotypic FH as a score above 5. [165] Fatal ventricular arrhythmias (FVA), defined as primary ventricular

fibrillation (VF) or sustained ventricular tachycardia (VT), were confirmed by reviewing rhythm strips and/or 12-lead electrocardiograms.

Among all subjects with premature AMI, the incidence of FVA was 4.7% (n=11). In total, 11.2% (n=26) of patients with early-onset AMI had probable/definite FH, with mean age 46.8±7.4 years and mean LDL-C 5.6±1.9 mmol/L versus 2.5±0.8 mmol/L in unlikely FH (n=104). There was no significant difference in the prevalence of diabetes (p=0.94), hypertension (p=0.87) or smoking (p=0.24) among the groups. Compared to patients with possible and unlikely FH, patients with likely FH showed significantly lower rates of FVA (4.3%, 0.9%, 0%, p=0.007). Left ventricular ejection fraction (LVEF) was not significantly different between the groups (p=0.11). On multivariable analysis, FH was independently associated with a lower frequency of FVA, adjusted for age, LVEF <40% and type of AMI (ST elevation vs non-ST elevation) [Odds ratio=0.65 (95% Confidence Interval 0.74 to 0.87), p=0.04].

In this modest size population based retrospective study, we found an independent negative association between FH, higher plasma LDL-C level and incidence of FVA complicating AMI. This result is consistent with the limited existing data linking higher cholesterol levels with a lower incidence of primary FVA in the setting of AMI. [2,3] Although the sarcolemmal lipid composition of myocytes is the main modulator of ion channel function and development of arrhythmias, [(163)] the precise mechanisms via which hypercholesterolemia may influence these effects remains an area of interest for future cardiac electerophysiological research. A limitation to our retrospective study is that we cannot determine the impact of unknown confounders resulting in primary VF. Furthermore, our data does not undermine the established strong

association between FH, hypercholesterolemia, and development of atherosclerosis and further AMI. [1]

In conclusion, we found FH and higher levels of LDL-C to be associated with a lower incidence of primary VF in patients with premature AMI independently of other risk factors. At present, there remains insufficient data to support the exact pathophysiological mechanism of higher death and poor prognosis in FH, however this study suggests that the mortality associated with AMI in patients with FH may be due to non-arrhythmic complications. Hence, continued study is necessary to identify the long term impacts of hyperholesterolaemia and the precise mechanisms for poor prognosis in this high risk population.

References

1. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease Consensus Statement of the European Atherosclerosis Society. European Heart Journal. 2013;34(45):3478-90.

2. Dekker LR, Bezzina CR, Henriques JP, Tanck MW, Koch KT, Alings MW, et al. Familial sudden death is an important risk factor for primary ventricular fibrillation: a case-control study in acute myocardial infarction patients. Circulation. 2006;114(11):1140-5.

3. Baartscheer A, Schumacher CA, Wekker V, Verkerk AO, Veldkamp MW, van Oort RJ, et al. Dyscholesterolemia Protects Against Ischemia-Induced Ventricular Arrhythmias. Circulation Arrhythmia and electrophysiology. 2015;8(6):1481-90.

4. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. The Lancet. 1999;354(9177):447-55.

5. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. International journal of cardiology. 2014;171(3):309-25.

Chapter 3 C

The Tertiary Hospital Laboratory; a Novel Avenue of Opportunistic Screening of Familial Hypercholesterolemia

IJC Heart & Vasculature 23 (2019) 100354

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vasculature

The tertiary hospital laboratory; a novel avenue of opportunistic screening of familial hypercholesterolemia

Sam Mirzaee [a,*], Kay W. Choy [b], James C.G. Doery [b], Sarah Zaman [a], James D. Cameron [a], Arthur Nasis [a]

[a] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia
[b] Monash Health Pathology, Monash Health, Monash University, Melbourne, Australia

ARTICLE INFO

Article history:
Received 6 January 2019
Received in revised form 9 March 2019
Accepted 24 March 2019
Available online 3 May 2019

Keywords:
Familial hypercholesterolemia
FH
Tertiary hospital laboratory
Opportunistic screening

ABSTRACT

Background: Familial hypercholesterolemia (FH) is a common monogenic hereditary lipid disorder characterised by increased serum low-density lipoprotein cholesterol (LDL-cholesterol) concentrations and high risk of premature atherosclerotic cardiovascular disease. The prevalence of FH identified in a tertiary hospital laboratory was investigated by performing an opportunistic screen for index cases.
Methods: The prevalence of likely FH based on LDL-cholesterol thresholds >4.9 mmol/L as employed by the Dutch Lipid Clinic Network Criteria (DLCNC) score was evaluated retrospectively in a single tertiary hospital laboratory over a six-month period (July to December 2016).
Results: 4943 lipid profiles screened, 106 patients (mean age 53.2 ± 12.9 and 41% male) had LDL-cholesterol of >4.9 mmol/L after exclusion of 5 patients (0.1%) with secondary causes. Possible (n = 90) and probable/definite (n = 16) FH according to DLCNC score was seen in 1.8% and 0.4% of the overall screened population, respectively.
Conclusions: Point prevalence of screening for FH in patients undergoing lipid profile testing in a tertiary hospital laboratory was comparable with prevalence of FH in general population (based on 1 in 200–250). This supports the benefit of establishing an efficient "alert system" in conjunction with a trigger "reflex testing" to facilitate further formal FH scoring and exclusion of possible secondary causes of hyperlipidemia in potential index FH.

1. Introduction

Familial hypercholesterolemia (FH) is a hereditary lipid disorder characterised by increased serum low-density lipoprotein cholesterol (LDL-cholesterol) concentrations and premature atherosclerotic cardiovascular disease (ASCVD) [1]. It is an autosomal dominant condition frequently caused by mutations in the gene coding for the LDL-receptor (LDL-R) and, less commonly, mutations in the genes coding for Apolipoprotein-B (APO-B) and proprotein convertase subtilisin/kexin type 9 (PCSK9) [2].

The prevalence of FH has previously been reported as 1 in 500 in the general population [3–5]. However, emerging evidence suggests that FH is potentially more common than and possibly as high as 1 in 200–250 in the general population, and is often both underdiagnosed and undertreated by health care providers [6,7]. The risk of early-onset ASCVD is increased tenfold among untreated individuals with FH. Statin therapies have been demonstrated to improve the prognosis of patients with FH [8]. Hence, FH guidelines focus on early identification and management of index cases as well as cascade screening to prevent further cardiovascular events [9]. The key role of biochemistry laboratories and chemical pathology in detecting index cases of FH has been pointed out in an Australasian model of care for FH [10]; however, there is no existing data to support this proposed role.

There are several published criteria for the diagnosis of phenotypical FH, with the Dutch Lipid Clinic Network Criteria (DLCNC) score appearing to have more comprehensive stratification criteria and higher sensitivity for identifying screened individuals with FH [11]. On the basis of LDL-cholesterol level, clinical examination, family history, and genetic testing the odds of having FH can be assessed via DLCNC: definite FH >8, probable FH 6–8, possible FH 3–5 and unlikely FH <3 [12]. As universal screening for FH is economically prohibitive, every opportunity to screen patients for FH should be utilised. The feasibility of community laboratories to screen for individuals with potential FH has been formerly studied and described [1]. We studied the potential viability of a tertiary hospital laboratory as a novel avenue to opportunistically screen for patients with FH. This avenue of identification has not been previously evaluated.

Abbreviations: AHA, American Heart Association; ASCVD, Atherosclerotic cardiovascular disease; APO-B, Apolipoprotein-B; CAD, Coronary artery disease; DLCNC, Dutch Lipid Clinic Network Criteria; FH, Familial hypercholesterolemia; HDL-C, High density lipoprotein cholesterol; HIV, Human immunodeficiency virus; LDL-cholesterol, Low-density lipoprotein cholesterol; LDL-R, Low density lipoprotein receptor; PCSK-9, Proprotein convertase subtilisin/kexin type-9.

* Corresponding author at: Monash Cardiovascular Research Centre, MonashHeart, Monash Health, 246 Clayton Road, Clayton, VIC 3150, Australia.
E-mail address: sam.mirzaee@monashhealth.org (S. Mirzaee).

https://doi.org/10.1016/j.ijcha.2019.100354

2. Methods

All fasting serum lipid profiles consecutively performed over six months (from 3rd July 2016 to 29th December 2016) from a single tertiary hospital pathology laboratory (Monash Health, Melbourne, Australia) were reviewed. Monash Health is Victoria's largest public health service providing care to a population of over 3.6 million people. Lipid profiles with no reported LDL-cholesterol or reported non-calculated due to high triglyceride levels were excluded. Total cholesterol, triglyceride and high-density lipoprotein (HDL)-cholesterol were performed with enzymatic, colorimetric assays on a Beckman Coulter AU 5800 analyser (Beckman Coulter, Brea, CA, USA). The inter- and intra-assay percent coefficients of variation (%CVs) of these assays were <5%. LDL-cholesterol was calculated according to the Friedewald equation [13]. Patients with a serum LDL-cholesterol >4.9 mmol/L (correlating with a DLCNC score of at least 3) were further assessed.

Electronic hospital medical records were reviewed for clinical and medication history to evaluate further components of DLCNC and known secondary causes of hypercholesterolemia including untreated hypothyroidism, nephrotic syndrome, anorexia nervosa and cholestasis. The use of relevant medications at the time of performed lipid profile was recorded. In patients receiving LDL lowering therapies, the LDL-cholesterol plasma level was adjusted according to the intensity and the dose of administered statin or ezetimibe [14]. The specialty of the medical practitioner who referred the patient for lipid profiling was also collected and analysed. Microsoft Excel 2013 and MedCalc statistical software version 16.4.3 was used for statistical analysis [15]. This research was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki), and our institutional review board approved the study (RES-17-0000-433Q).

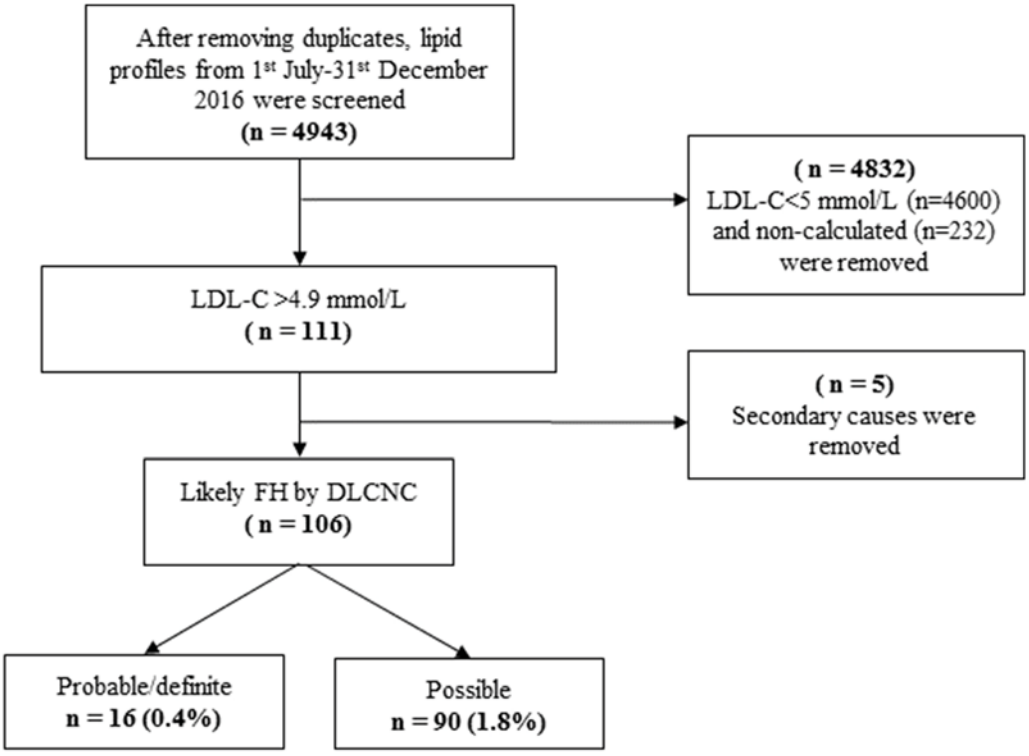
3. Results

Over the 6-month period, a total of 4943 serum lipid profiles were reviewed. There were 106 (2.2%) subjects with LDL-cholesterol >4.9 mmol/L after exclusion of five cases (0.1%) with identified secondary causes [untreated hypothyroidism (n = 1), severe renal failure (n = 2), cholestasis (n = 1), anti-HIV (human immunodeficiency virus) medication (n = 1)]. After review of 4943 electronic pathology result form, 1.8% of all patients who had LDL-cholesterol measured over the 6 months had a DLCNC score of 3–5, 0.3% scored 6–8 and 0.1% scored >8 indicating a possible, probable and definite diagnosis of FH respectively (Fig. 1). We adjusted the prevalence of possible FH to 0.03% accordingly. In total, the point prevalence of likely phenotypical FH based on DLCNC and a LDL-cholesterol >4.9 mmol/L was calculated as approximately 0.43% (1 in 232).

The serum LDL-C distribution is shown in Fig. 2, and the basic characteristics of individuals with likely FH are summarised in Table 1. The 95th, 99th and 99.75th percentiles for serum LDL-cholesterol were 4.4, 5.5 and 6.4 mmol/L, respectively. None of these patients had a previously documented diagnosis of FH according to the medical record. The vast majority of these patients with likely FH and elevated LDL-cholesterol were statin naïve (85.9%). Patients were referred for lipid profiling by specialists in 63% (cardiologists were 21% of specialist's referrals) of cases and by general practitioners in 37% with the majority of these initiated after recent hospital admission.

4. Discussion

The current study demonstrated an LDL-cholesterol >4.9 mmol/L with DLCNC score consistent with probable/definite or possible FH



DLCNC: Dutch Lipid Clinic Network Criteria; FH: familial hypercholesterolemia; LDL-C: Low Density Lipoprotein Cholesterol

Fig. 1.. Patient selection and study design. Diagram demonstrating study inclusion and exclusion criteria.

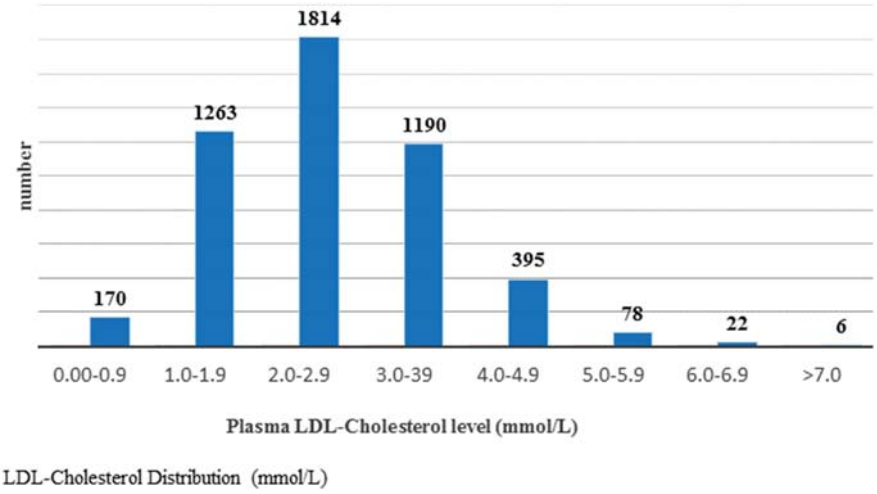


Fig. 2.. Plasma LDL-cholesterol distribution in a tertiary laboratory.

was seen with a prevalence of 1:50 in our screened population. Our results on the basis of DLCNC screening are significantly higher than the approximately 1:200–250 proposed in the general population. A recent large case-control study suggested that about 2% of individuals with elevated LDL-C > 4.9 mmol/L have an FH genetic mutation [16], if this result is applicable to our population of possible FH identified by DLCNC criteria (i.e. 2% of 1.8%), a final adjusted prevalence of approximately 0.43% (1 in 232) results. This is comparable to the proposed prevalence in the general population (1 in 200–250).

We anticipate that by applying genetic mutation adjustment, the actual prevalence of diagnosed FH in the tertiary hospital might be underestimated. Firstly, overall patients referred to a tertiary laboratory have more serious co-morbidities than the general population, so there is a higher likelihood of FH patients requiring hospitalisation. Secondly, the specificity of LDL-cholesterol cut-off point of 4.9 mmol/L may have been reduced by unknown confounders in this population. Finally, due to poor correlation (only ~40%) between mutation and a clinical diagnosis of FH [2], by using adjusted prevalence rate for possible FH, some cases with FH may have been excluded.

Although there is no other data regarding the prevalence of FH identified in tertiary laboratory screening, our results support previous studies that demonstrated a beneficial role of LDL-cholesterol cut-offs in screening for FH, in the absence of genetic testing [17]. Also, a serum LDL-cholesterol of >4.9 mmol/L in adults over 16 years of age is the cornerstone of laboratory diagnosis of possible FH by the Simon Broome criteria [18] as an alternative method for FH diagnosis. MED-PED criteria is another assessment tool to identify patients with FH; however, this would be more challenging to use for screening as it requires age adjustment in conjunction with plasma LDL-cholesterol cut-off levels [19]. The suitability of each diagnostic criterion versus genetic testing in a specific target population has been studied in one of the countries located in the Persian Gulf region [20].

Due to several limitations such as suboptimal documentation of medical records, ethnicity variation and underdiagnosed secondary hypercholesterolemia, determination of the exact prevalence of FH in a tertiary laboratory or even in the clinical setting remains challenging. Using an LDL-cholesterol cut off level criteria at least raises the possible presence of this preventable high-risk condition and prompts clinicians to explore for other diagnostic criteria. Furthermore, the fact that there is a weak correlation between positive FH genetic testing and clinical diagnostic criteria, the role of LDL-cholesterol cut-off level in screening for patients with FH will be more prominent [21,22]. Considering the described caveats and also the absence of consensus about the best diagnostic criteria to identify patients with phenotypical FH, the feasibility of screening FH via a tertiary laboratory using solely LDL-cholesterol cut-offs appears reasonable.

The majority of referrers for tertiary hospital lipid profile testing in our study were specialists particularly cardiologists; thus this study also highlights the importance of awareness and knowledge about FH among cardiologists in promoting management in this high-risk group. However, numerous referrals from high risk hospitalised patients might limit the validity of our findings when applied to a less homogenous and lower risk population. The fact that these index cases identified with probable or possible FH had not been diagnosed and therefore a vast majority of them were statin naïve illustrates that much education is still required of specialists and general practitioners in detecting FH. It also supports that laboratories performing lipid profiles should create a routine "alert system" for all LDL-cholesterol >4.9 mmol/L to the requesting doctor to ensure further screening for FH or referral to a lipid specialist is considered.

Table 1
Characteristics of patients with likely FH (n = 106).

Characteristics	Probable/definite (n = 16)	Possible (n = 90)
Age, y ± SD	53.3 ± 12.4 (38–66)	53.2 ± 13.0 (14–82)
Male	7 (43%)	36 (40%)
Total cholesterol (mmol/L)	7.9 ± 0.8	7.9 ± 0.8
LDL-C (mmol/L)	5.7 ± 0.6	5.6 ± 0.6
Diabetes %	5 (31%)	7 (8%)
Hypertension %	3 (27%)	10 (11%)
Smoking %	5 (31%)	4 (5%)
Family history of early ASCVD %	7 (63%)	4 (5%)
Peripheral arterial disease	1 (%6)	1 (1%)
DLCNC mean ± SD	7.7 ± 0.5	3.1 ± 0.5
Ischemic heart disease %	11 (69%)	8 (9%)
STEMI	3 (19%)	1 (1%)
Non-STEMI	5 (31%)	4 (5%)
Unstable angina	3 (19%)	3 (3%)
Stroke/TIA %	1 (6%)	2 (2%)
Use of statins	14 (13.2%)	1 (1%)

ASCVD: Atherosclerotic Cardiovascular Disease; DLCNC: Dutch Lipid Clinic Network Criteria; FH: familial hypercholesterolemia; LDL-C: Low Density Lipoprotein Cholesterol; SD: Standard deviation; STEMI: ST Elevation Myocardial Infarction; TIA: Transient Ischemic Attack.

5. Limitations

This was a retrospective study screening patients mainly by existing LDL-cholesterol levels in the biochemistry laboratory. In cases associated with high triglycerides (above 4.5 mmol/L) and also some patients with combined hyperlipidemia, calculated LDL-cholesterol level was

diluted due to erroneously applied Freidewald's formula [23]. Conversely, patients with lower LDL-cholesterol level may have other clinical manifestation of FH which would be underdiagnosed by the use of solely biochemical criteria. There was no genetic testing information in this FH detected patients, which may provide an additional diagnostic and prognostic value to this high-risk subgroup. We cannot describe whether the higher rate of FH in a tertiary hospital laboratory is related to lower specificity of screening or if this is a selection bias due to a high frequency of referral of severe cases such as acute coronary syndromes, stroke or peripheral arterial disease to a tertiary centre.

6. Conclusions

This study demonstrates a high yield from opportunistic screening for FH in a tertiary hospital laboratory with a prevalence of at least analogous to the general population. The detection of previously undiagnosed and undertreated cases of likely FH supports the benefit of establishing an efficient “alert system” in tertiary laboratories to facilitate screening and referral to a lipid specialist in this high-risk population.

Funding

This project was not supported by an external grant.

Conflict of interest statement

SM received an honorarium from Amgen.

References

[1] D.A. Bell, A.J. Hooper, R. Bender, J. McMahon, G. Edwards, F.M. van Bockxmeer, et al., Opportunistic screening for familial hypercholesterolaemia via a community laboratory, Ann. Clin. Biochem. 49 (2012) 534–537 Pt 6.
[2] M. Sharifi, M. Futema, D. Nair, S.E. Humphries, Genetic architecture of familial hypercholesterolaemia, Curr. Cardiol. Rep. 19 (5) (2017) 44.
[3] D. Marks, M. Thorogood, H.A. Neil, S.E. Humphries, A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia, Atherosclerosis 168 (1) (2003) 1–14.
[4] M.A. Austin, C.M. Hutter, R.L. Zimmern, S.E. Humphries, Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review, Am. J. Epidemiol. 160 (5) (2004) 407–420.
[5] R. Bender, D.A. Bell, A.J. Hooper, G. Edwards, F.M. van Bockxmeer, G.F. Watts, et al., Screening for familial hypercholesterolaemia, Pathology. 44 (2) (2012) 122–128.
[6] G.F. Watts, S. Gidding, A.S. Wierzbicki, P.P. Toth, R. Alonso, W.V. Brown, et al., Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation, Int. J. Cardiol. 171 (3) (2014) 309–325.
[7] P. Faggiano, A. Pirillo, R. Griffo, M. Ambrosetti, R. Pedretti, G. Scorcu, et al., Prevalence and management of familial hypercholesterolemia in patients with coronary artery disease: the heredity survey, Int. J. Cardiol. 252 (2018) 193–198.
[8] J. Versmissen, D.M. Oosterveer, M. Yazdanpanah, J.C. Defesche, D.C. Basart, A.H. Liem, et al., Efficacy of statins in familial hypercholesterolaemia: a long term cohort study, Br. Med. J. (Clin. Res. Ed.) 337 (2008) a2423.
[9] G.F. Watts, D.R. S0075llivan, N. Poplawski, F. van Bockxmeer, I. Hamilton-Craig, P.M. Clifton, et al., Familial hypercholesterolaemia: a model of care for Australasia, Atheroscler. Suppl. 12 (2) (2011) 221–263.
[10] G.F. Watts, D.R. Sullivan, F.M. van Bockxmeer, N. Poplawski, I. Hamilton-Craig, P.M. Clifton, et al., A model of care for familial hypercholesterolaemia: key role for clinical biochemistry, Clin. Biochem. Rev. 33 (1) (2012) 25–31.
[11] D.G. Shin, S.M. Han, D.I. Kim, M.Y. Rhee, B.K. Lee, Y.K. Ahn, et al., Clinical features of familial hypercholesterolemia in Korea: predictors of pathogenic mutations and coronary artery disease - a study supported by the Korean Society of Lipidology and Atherosclerosis, Atherosclerosis 243 (1) (2015) 53–58.
[12] , World Health Organisation Familial Hypercholesterolaemia (FH): Report of a Second WHO Consultation. , World Health Organization, Geneva, 1999 (World Health Organization).
[13] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502.
[14] Dutch Lipid Clinical Network Score (DLCNS) Online Calculator© 2017 FH Australasia Network: Available from: https://www.athero.org.au/fh/calculator/.
[15] MedCalc Statistical Software Version 16.4.3. , MedCalc Software bvba, Ostend, Belgium, 2016https://www.medcalc.org.
[16] A.V. Khera, H.H. Won, G.M. Peloso, K.S. Lawson, T.M. Bartz, X. Deng, et al., Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia, J. Am. Coll. Cardiol. 67 (22) (2016) 2578–2589.
[17] B. Starr, S. Gaye Hadfield, B.A. Hutten, P. Lansberg, T.P. Leren, D. Damgaard, et al., Development of Sensitive and Specific Age- and Gender-Specific Low-Density Lipoprotein Cholesterol Cutoffs for Diagnosis of First-Degree Relatives With Familial Hypercholesterolaemia in Cascade Testing, 2008 (791–803 p.).
[18] H. Neil, D. Betteridge, K. Broome, P. Durrington, M. Hawkins, S. Humphries, et al., Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group, Atherosclerosis 142 (1) (1999) 105–112.
[19] R.R. Williams, S.C. Hunt, M.C. Schumacher, R.A. Hegele, M.F. Leppert, E.H. Ludwig, et al., Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics, Am. J. Cardiol. 72 (2) (1993) 171–176.
[20] K. Al Rasadi, K. Al-Waili, H. Al-Sabti, A. Al-Hinai, K. AlHashmi, I. Al-Zakwani, et al., Criteria for Diagnosis of Familial Hypercholesterolemia: A Comprehensive Analysis of the Different Guidelines, Appraising their Suitability in the Omani Arab Population, 2014 (85–91 p.).
[21] M. Sharifi, M. Futema, D. Nair, S.E. Humphries, Genetic architecture of familial hypercholesterolaemia, Curr. Cardiol. Rep. 19 (5) (2017) 44.
[22] K. Haralambos, S.D. Whatley, R. Edwards, R. Gingell, D. Townsend, P. Ashfield-Watt, et al., Clinical experience of scoring criteria for Familial Hypercholesterolaemia (FH) genetic testing in Wales, Atherosclerosis. 240 (1) (2015) 190–196.
[23] C.M.M. de Cordova, M.M. de Cordova, A new accurate, simple formula for LDL-cholesterol estimation based on directly measured blood lipids from a large cohort, Ann. Clin. Biochem. 50 (1) (2013) 13–19.

Chapter 4

Assessment of vascular inflammation by Pericoronary Adipose Tissue Attenuation Using Computed Tomography in Patients with Familial Hypercholesterolemia

CHAPTER 4

Preface

Patients with heterozygous familial hypercholesterolemia (FH) have substantial higher risk of early onset cardiovascular disease (CVD) and 2-fold greater risk of recurrent cardiovascular events which results in poorer prognosis in this high risk of population.[1] Nevertheless, there is heterogeneity in phenotypical expression and development of cardiovascular disease among FH patients with substantial individual variation. In the next two chapters we have focused on investigating FH patients with CVD and without CVD to risk stratify and identifying novel parameters which will impact on development of CVD within this high risk group. Inflammation has been described as a key feature in the genesis of CVD, and imaging modalities that can detect vascular inflammation would allow more precise non-invasive cardiovascular risk stratification.[2] Therefore, we sought to assess vascular inflammation by a novel parameter peri-coronary adipose tissue (PCAT) attenuation, using computed tomography. We hypothesised vascular inflammation is higher in patients with FH and CVD compared to without CVD.

References

1. Nanchen D, Gencer B, Muller O, Auer R, Aghlmandi S, Heg D, et al. Prognosis of Patients With Familial Hypercholesterolemia After Acute Coronary Syndromes. Circulation. 2016;134(10):698-709.

2. Oikonomou EK, Marwan M, Desai MY, Mancio J, Alashi A, Hutt Centeno E, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet 2018;392:929–939

Assessment of Vascular Inflammation by Pericoronary Adipose Tissue Attenuation using Computed Tomography in patients with familial hypercholesterolemia

Sam Mirzaee [1], Udit Thakur[1], Andrew Lin[1], Nitesh Nerlekar[1], James D Cameron [1], Arthur Nasis, Stephen J Nicholls[1], Dennis TL Wong[1]

[1] Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Monash University, Melbourne, Australia

Corresponding Author:

Associate Professor Dennis Wong

Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Victoria, Australia

Email: dennis.wong@monash.edu

Tel: +61 3 9695 6666

Fax: +61 3 9594 4246

ABSTRACT

Introduction: Heterozygous Familial Hypercholesterolemia (HeFH) is a common hereditary lipid disorder which causes premature coronary artery disease. Pericoronary adipose tissue (PCAT) attenuation is a novel computed tomography coronary angiography (CTCA) biomarker of coronary inflammation with prognostic validation. We sought to compare PCAT attenuation in HeFH patients with and without significant coronary artery disease (CAD).

Methods: Patients with probable/definite FH as per Dutch Lipid Clinical Network Criteria who underwent 320-detector CTCA were included in this cross-sectional study. Above 50% coronary stenoses in CTCA defined as significant CAD. Using semi-automated software (Autoplaque version 2.0), PCAT attenuation was measured around the proximal 10-50 mm of the right coronary artery (RCA). In patients with significant CAD, PCAT attenuation was measured around the lesion with highest-grade stenosis.

Results: Sixty patients with phenotypic FH (40 with significant CAD and 20 with no CAD) were included. Median low-density lipoprotein cholesterol (LDL-C) was higher in patients without CAD compared to those with significant CAD (5.3 [IQR 3.8-6.7] vs. 3.3 [2.6- 4.5] mmol/L, p<0.001]. PCAT attenuation was significantly higher in patients with significant CAD compared to those without CAD (-80.9 ± 12.2 vs. -90.6 ± 7.6 HU, *p*=0.004). PCAT attenuation around the proximal RCA was not statistically significant between the two groups (-85.3 ± 11.9 vs. -90.6 ± 7.6, p=0.09). PCAT attenuation did not correlate with plasma LDL-C levels.

Conclusion: HeFH patients with significant CAD had a higher PCAT attenuation than those without CAD, perhaps reflecting a greater degree of coronary inflammation. Future studies are required to assess whether PCAT attenuation can enhance risk stratification and guide therapy in FH patients. **Key words:** Familial hypercholesterolemia, Coronary artery disease, Pericoronary Adipose Tissue Attenuation, CT coronary angiography

INTRODUCTION

Heterozygous Familial hypercholesterolemia (HeFH) is a common hereditary disorder of lipoprotein metabolism characterised by high plasma concentrations of low-density lipoprotein cholesterol (LDL-C), and a high risk of premature atherosclerotic cardiovascular disease (ASCVD) (1). Current data suggests patients with FH have over a 10-fold higher risk of early onset ASCVD and 2-fold greater risk of recurrent cardiovascular events which results in poorer prognosis in this high risk of population (1, 2). Nevertheless, there is heterogeneity in phenotypical expression and development of ASCVD among FH patients with substantial individual variation.

Risk of developing cardiovascular disease in HeFH could be multifactorial due to different genetic composition, gender, body mass index (BMI), hypertension, smoking, insulin resistance and possibly other coexisting forms of lipid disorders particularly elevated lipoprotein (a) [Lp(a)] (3, 4). Coronary computed tomographic angiography (CTA) and vascular imaging is a useful tool to detect and quantify the course of atherosclerosis, in addition to analyse the plaque composition characteristics for further risk stratification in this high risk of population (5-7).

Inflammation has been described as a key feature in genesis of ASCVD, and imaging modalities that can detect vascular inflammation would allow more precise cardiovascular risk stratification (8). More recently, pericoronary Adipose Tissue (PCAT) attenuation by Computed Tomography (CT) has been proposed as a marker for assessment of qualitative heterogeneity in coronary segments (9, 10). PCAT attenuation has been shown to be increased around ruptured atherosclerotic plaques in patients acutely during myocardial infarction (8). Paracrine signals originating from the vascular wall alter the fat attenuation of the PCAT surrounding it (8). Due to these localised signalling pathways, PCAT attenuation is associated with vessel inflammation and

plaque burden, independent to systematic metabolic risk factors (8). However, there is limited information describing the prognostic value association of PCAT in FH patients.

Our goals in this study were to evaluate PCAT attenuation by using coronary CTA in FH subjects with and without significant CAD, to identify the association of this novel parameter with coronary plaques and their composition to better stratify their cardiovascular risk.

METHODS

Study Population

This retrospective, single-centre case control study evaluated a total of 60 patients with probable/definite FH using a modified version of the Dutch Lipid Clinic Network Criteria (DLCNC) and ≥18 years of age from February 2017 to December 2018 at MonashHeart (Monash Health, Melbourne, Australia). Patients had to have underwent a clinically indicated CTCA 320-detector CTCA for suspected CAD. Twenty four patients with DLCN<6, and inadequate image quality were excluded. Basic characteristics and conventional cardiovascular risk factors, such as hypertension, diabetes, smoking and obesity, were obtained from the hospital electronic medical records. Missing clinical data were collected prospectively by phone interview of subjects. The study was approved by the Human Research and Ethics Committee of Monash Health (HREC/RES-17-0000-425Q).

CTCA Acquisition and Interpretation

All CTCAs were performed on a 320-detector-row scanner (Aquilion Vision, Toshiba, Japan) at MonashHeart. The image acquisitions were performed according to departmental protocol (11) and established guidelines (12). All studies were previously reported by two expert readers unaware of

prospect of FH. The presence of significant CAD was defined as the presence of a lesion visually graded as having stenosis ≥50% of luminal diameter in a vessel of greater than 2mm diameter.

Coronary plaque analysis via semi-automated software

An experienced reader blinded to the patient data, completed the plaque analysis within the proximal right coronary artery (RCA) in addition to all other segments with visible atheroma and a luminal diameter of greater than 2mm using semi-automated software (Autoplaque, version 2.0; USA Cedar-Sinai Medical Center, Los Angeles, CA). The proximal RCA (10–50 mm from RCA ostium) has been used in former studies as a standard model for PCAT analysis (13, 14).

Figure 1 demonstrates plaque analysis of a patient with significant proximal RCA disease compared to a patient without. Transverse and multiplanar reconstruction (MPR) views were used to assess the CT images. In MPR views, a region of interest was placed in the aorta to define blood pool and 5–7 control points were positioned in the arterial lumen to define central line. The proximal and distal limits of the proximal RCA were identified as previously described. Subsequently, we used the same approach for lesion analysis of the segmental coronary disease with semi-automated vessel wall correction as well as fine adjustment of outer vessel wall. Per-case processing time for analysis was variable ranged between 30-60 min depending on the number of lesions, characteristics of the lesion and image quality. After plaque quantification PCAT assessment was computerized.

Analysis of peri coronary adipose tissue attenuation

PCAT was fully automated by computerised measure of three-dimensional layers, starting from the outer vessel wall increasing 1 mm cylindrical layer outwards. Adipose tissue was defined as all voxels with attenuation between -190 HU and -30 HU, and the PCAT CT attenuation was defined as the average CT attenuation in HU of the adipose tissue within the defined volume of interest (Figure 2).(8, 10) As recently described 3 mm average luminal diameter of the proximal RCA, we considered the PCAT CT attenuation (HU) within a volume between the vessel wall and an outer radial distance of 3 mm from the vessel wall (13). Figures 2A-D show PCAT quantification of the proximal RCA and a lesion analysis and in two patient examples with CAD disease and without. PCAT CT attenuation was exported for each proximal RCA in control group and lesion analysis along with proximal RCA in case group for comparison. Those patients who had RCA diameter less than 2mm or previously stented were excluded (n=15).

Epicardial adipose tissue measurement

Epicardial adipose tissue (EAT) was analysed by two independent operators as previously described methods (15). EAT volume and density was quantified from non-contrast CT using semi-automated software (QFAT version 2.0 software, Cedars-Sinai Medical Center, Los Angeles, CA, USA; (Figure 3). Pulmonary artery bifurcation was used as the superior landmark and the level of the posterior descending artery as the lower limit of the heart. Subsequently, the pericardium was manually traced 10 region of interest (ROI) aligning in different level of cardiac axis in the non-contrast. CT attenuation (HU) were automatically calculated by including contiguous 3D fat voxels between the HU limits of (-190, -30) enclosed by the visceral pericardium.

Laboratory Analyses

Lipid profile including total cholesterol, triglyceride and high-density lipoprotein (HDL)-cholesterol were performed with enzymatic, colorimetric assays on a Beckman Coulter AU 5800 analyser (Beckman Coulter, Brea, CA, USA). The inter- and intra-assay percent coefficients of variation (%CVs) of these assays were <5 %. LDL-cholesterol was calculated according to the Friedewald equation (16).

Ascertainment of phenotypical familial hypercholesterolemia

We used the modified DLCNC for diagnosis of phenotypical FH defined as the numerical sum to each of the criteria: definite, score >8; probable, score 6–8; possible, score 3–5; and unlikely, score <3. (17) Simplified DLCNC include: personal history of early onset CAD (at age <55 years for men and <60 years for women (2 points), personal history of stroke (1 point); premature family history of CAD (1 point); and plasma LDL cholesterol >8.5 mmol/L (8 points), LDL cholesterol 6.5–8.4 (5 points), LDL cholesterol 5–6.4 mmol/L (3 points), LDL cholesterol 4–4.9 mmol/L (1 point). In case of being on lipid lowering therapy, plasma LDL-C level was corrected according to the potency and dose of specific statins or ezetimibe to estimate pre-treatment levels (18). In this study, phenotypic FH was defined as a score >5 (probable/definite FH).

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median and interquartile range in the case of non-normal distribution. The Shapiro–Wilk test was used to assess normality for continuous data. Chi square or Fisher exact test used for comparison of categorical variables, and two-sample t-test or Wilcoxon rank-sum test were applied to evaluate the differences between continuous variables. Statistical significance was defined as $p < 0.05$ (two-tailed). PCAT

measurements were indexed by time between scans. Statistical analyses was performed with STATA-14 (StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics

The study population comprised of 60 patients (40 with significant CAD and 20 without CAD), with a mean age of 47.8±11.03 vs 47.5±6.8 years and 82% vs 20% males, respectively. Baseline patient characteristics are presented in Table-1.The prevalence of existing statin therapy was not significantly different in patients with or without disease compared to those with 47% vs15%, *p*=0.082). However, the median LDL-C level was significantly higher in control group [3.3(2.6-4.5) vs 5.3 (3.8-6.7), *p*<0.001].

Per-group pericoronary adipose tissue attenuation comparing global vs lesion

Whereas comparing the PCAT in control (global) vs. the PCAT in cases using per lesion measurement based on mean luminal diameter (MLD), there is a statistically significant difference between cases and control group -80.9 HU ± 12.2 vs. -90.6 HU ± 7.6, *p*=0.004, respectively (Table- 2).

Per-group pericoronary adipose tissue attenuation comparing global vs global

PCAT attenuation index in FH Patients without CAD in comparison with those with significant CAD did not show statistically significant difference -85.3 HU ± 11.9 vs. -90.6 HU ± 7.6, *p*=0.09 (Table- 2).

Epicardial fat attenuation comparison

When evaluating the EAT density in FH patients with CAD compared to those without CAD, there was no statistically significant difference -87.7 HU +/- 6.2 vs -86.9 HU +/- 7.1, p=0.10 (Table 2).

Peri coronary adipose tissue attenuation correlation with Lipid parameters

When correlating global PCAT attenuation with LDL-C, a very weak linear relationship was seen (r^2=0.2). Similarly, there was no meaningful correlation between PCAT attenuation using per lesion measurement with LDL-C (r^2=0.1).

DISCUSSION

The present analysis is the first to demonstrate a relationship between lesion vascular inflammation using imaging perivascular fat and the presence of significant coronary atherosclerotic plaque assessed by CTCA in patients with phenotypical FH. Other findings of the present study were: 1) general vascular inflammation between two groups of FH patient was not correlated indicating more local effect of PCAT rather than systemic influence. This is in keeping with the results from the EAT comparison between the two cohorts. Given there was no statistically significant difference between the cases and controls, this suggests that there isn't a global effect rather sections of the coronary tree are inflamed in FH patients. 2) The level of PCAT attenuation index was not correlated with LDL-C levels.

Former studies demonstrated that the surrounding adipose tissue imply paracrine effects on the adjacent vascular segments, which can be the predictor of cardiovascular events by using a non-

invasive CT imaging (8). Our data is in line with growing evidence showing the role of PCAT in focal vascular disease and long-term cardiovascular outcomes including all-cause mortality and cardiac events beyond clinical risk factors (19, 20). Interestingly, Ohyama et al. demonstrated that perivascular inflammation is associated with impaired vascular function causing coronary spasm which may explain the cardiac symptoms in our FH group without significant angiographic disease (21).

There is a large body of epidemiological evidence showing a positive correlation between LDL-C and development of ASCVD in FH (1). However, not all patients with FH develop ASCVD and heterogeneity has been reported among FH patients associated with several modifiable and non-modifiable risk factors (lifestyle, environmental, genetics, nutrition, etc.) that influence the course of ASCVD in this high risk population. (22) On the other hand, cholesterol as an unsaturated alcohol of the steroid family has various biological functions as a precursor molecule for several biochemical pathways that may have an inverse effect on systemic inflammation (23). We have also inversely found a higher LDL-C level in patients with FH and no coronary disease which can be postulated this may be related to their compliance with lipid lowering therapy and diet. However, this may support the role of novel risk factors such as anti-oxidant and inflammatory biomarkers in development of ASCVD rather than the quantity of LDL-C alone (24).

There is existing information indicating circulating inflammatory biomarkers such as high sensitivity C-reactive protein (hs-CRP) are unspecific for coronary inflammation (25), therefore a more specific non-invasive measurement of coronary inflammation to better risk stratify ASCVD events is an unmet need. Recently, Oikonomou et al. reported coronary inflammation mapping using PCAT attenuation index in coronary CT with a cut-off value ≥–70·1 HU, and suggesting this value enhances cardiac risk prediction and re-stratification in both primary and secondary

prevention (14). In our study, global PCAT attenuation index as a signal of coronary inflammation was higher than ≥–70·1 HU in all individuals with or without disease, indicating a greater risk of ASCVD in general. This is consistent with current literature demonstrating individual with FH have higher level of subclinical inflammation and subsequently have a significant (3- to 13-fold) greater risk of premature ASCVD compared with those without FH (26). Since traditional risk stratification tools were not accurate in patients with FH (27), SAFEHEART study introduced a novel risk stratification tool for FH patients (26). We suggest including a non-invasive coronary inflammation value such as the PCAT attenuation index may enhance the SAFEHEART risk equation in patients with FH.

Study limitations

There are several limitations to this study. Firstly, this was a retrospective trial of consecutive patients with familial hypercholesterolemia who had previously performed a CTCA. The time of image acquisition was variable among patients with significant coronary disease, and data about confounding effects from the use of adjuvant therapies such as anti-inflammatory medications or other lipid lowering agents on coronary plaque were not equally available. Secondly, we used the modified DLCNC for phenotypical diagnosis of FH. Although specificity is not as precise with the use of DLCNC compared to individual genetic testing, the advantage of using the modified DLCNC is its high sensitivity and suitability for screening. Thirdly, we performed this study on 60 patients with FH which is likely under powered. Moreover, the assessment of vascular inflammation may be potentially underestimated with the use by PCAT. Finally, we did not perform coronary artery calcium (CAC) scoring in this primarily symptomatic study population undergoing CTCA for suspected CAD. The clinical use of CAC scoring is in an asymptomatic population and hence was not applicable to our study cohort.

CONCLUSION

There is a significant association between lesion PCAT attenuation index and segmental atherosclerotic disease assessed non-invasively by CTCA which did not correlate with LDL-C levels, independent of HDLC and traditional CV risk factors. Future studies examining whether lowering of segmental atheroma burden can modulate lesion vascular inflammation and reduce the substantial residual CV risk seen after plaque stabilisation are warranted.

ACKNOWLEDGEMENTS

None.

SOURCES OF FUNDING

SM is supported by a Postgraduate Scholarship from the Australian Postgraduate Award. AL is supported by a Postgraduate Scholarship from the National Health and Medical Research Council. DW is supported by a National Health and Medical Research Council Early Career Fellowship. SN is supported by a National Health and Medical Research Council Principal Research Fellowship.

DISCLOSURES

SM received consultation fees from Amgen. DW has received speaking fees from Bayer, AstraZeneca, Novartis, Merck Sharp & Dome and Boehringer Ingelheim.

References

1. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease : Consensus Statement of the European Atherosclerosis Society. European Heart Journal. 2013;34(45):3478-90.

2. Nanchen D, Gencer B, Muller O, Auer R, Aghlmandi S, Heg D, et al. Prognosis of Patients With Familial Hypercholesterolemia After Acute Coronary Syndromes. Circulation. 2016;134(10):698-709.

3. Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nature clinical practice Cardiovascular medicine. 2007;4(4):214-25.
4. Santos RD. Phenotype vs. genotype in severe familial hypercholesterolemia: what matters most for the clinician? Current opinion in lipidology. 2017;28(2):130-5.

5. Nicholls SJ, Hsu A, Wolski K, Hu B, Bayturan O, Lavoie A, et al. Intravascular ultrasound-derived measures of coronary atherosclerotic plaque burden and clinical outcome. Journal of the American College of Cardiology. 2010;55(21):2399-407.

6. Bittencourt MS, Hulten E, Ghoshhajra B, O'Leary D, Christman MP, Montana P, et al. Prognostic value of nonobstructive and obstructive coronary artery disease detected by coronary computed tomography angiography to identify cardiovascular events. Circulation Cardiovascular imaging. 2014;7(2):282-91.

7. Perez de Isla L, Alonso R, Muniz-Grijalvo O, Diaz-Diaz JL, Zambon D, Miramontes JP, et al. Coronary computed tomographic angiography findings and their therapeutic implications in asymptomatic patients with familial hypercholesterolemia. Lessons from the SAFEHEART study. Journal of clinical lipidology. 2018;12(4):948-57.

8. Antonopoulos AS, Sanna F, Sabharwal N, Thomas S, Oikonomou EK, Herdman L, et al. Detecting human coronary inflammation by imaging perivascular fat. Science translational medicine. 2017;9(398).

9. Marwan M, Hell M, Schuhback A, Gauss S, Bittner D, Pflederer T, et al. CT Attenuation of Pericoronary Adipose Tissue in Normal Versus Atherosclerotic Coronary Segments as Defined by Intravascular Ultrasound. Journal of computer assisted tomography. 2017;41(5):762-7.
10. Goeller M, Achenbach S, Cadet S, Kwan AC, Commandeur F, Slomka PJ, et al. Pericoronary Adipose Tissue Computed Tomography Attenuation and High-Risk Plaque Characteristics in Acute Coronary Syndrome Compared With Stable Coronary Artery Disease. JAMA cardiology. 2018;3(9):858-63.

11. Wong DT, Soh SY, Ko BS, Cameron JD, Crossett M, Nasis A, et al. Superior CT coronary angiography image quality at lower radiation exposure with second generation 320-detector row CT in patients with elevated heart rate: a comparison with first generation 320-detector row CT. Cardiovascular diagnosis and therapy. 2014;4(4):299-306.

12. Abbara S, Arbab-Zadeh A, Callister TQ, Desai MY, Mamuya W, Thomson L, et al. SCCT guidelines for performance of coronary computed tomographic angiography: A report of the Society of Cardiovascular Computed Tomography Guidelines Committee. Journal of Cardiovascular Computed Tomography. 2009;3(3):190-204.

13. Goeller M, Tamarappoo BK, Kwan AC, Cadet S, Commandeur F, Razipour A, et al. Relationship between changes in pericoronary adipose tissue attenuation and coronary plaque burden quantified from coronary computed tomography angiography. European heart journal cardiovascular Imaging. 2019.

14. Oikonomou EK, Marwan M, Desai MY, Mancio J, Alashi A, Hutt Centeno E, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet (London, England). 2018;392(10151):929-39.

15. Goeller M, Achenbach S, Marwan M, Doris MK, Cadet S, Commandeur F, et al. Epicardial adipose tissue density and volume are related to subclinical atherosclerosis, inflammation and major adverse cardiac events in asymptomatic subjects. J Cardiovasc Comput Tomogr. 2018;12(1):67-73.

16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502.

17. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. International journal of cardiology. 2014;171(3):309-25.

18. Haralambos K, Whatley SD, Edwards R, Gingell R, Townsend D, Ashfield-Watt P, et al. Clinical experience of scoring criteria for Familial Hypercholesterolaemia (FH) genetic testing in Wales. Atherosclerosis. 2015;240(1):190-6.

19. Brown NK, Zhou Z, Zhang J, Zeng R, Wu J, Eitzman DT, et al. Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. Arteriosclerosis, thrombosis, and vascular biology. 2014;34(8):1621-30.

20. Chang L, Milton H, Eitzman DT, Chen YE. Paradoxical roles of perivascular adipose tissue in atherosclerosis and hypertension. Circulation journal : official journal of the Japanese Circulation Society. 2013;77(1):11-8.

21. Ohyama K, Matsumoto Y, Takanami K, Ota H, Nishimiya K, Sugisawa J, et al. Coronary Adventitial and Perivascular Adipose Tissue Inflammation in Patients With Vasospastic Angina. Journal of the American College of Cardiology. 2018;71(4):414-25.

22. Chan DC, Watts GF. Postprandial lipoprotein metabolism in familial hypercholesterolemia: thinking outside the box. Metabolism: clinical and experimental. 2012;61(1):3-11.

23. Tsoupras A, Lordan R, Zabetakis I. Inflammation, not Cholesterol, Is a Cause of Chronic Disease. Nutrients. 2018;10(5).

24. Papandreou C, Tuomilehto H. Coronary heart disease mortality in relation to dietary, lifestyle and biochemical risk factors in the countries of the Seven Countries Study: a secondary dataset analysis. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2014;27(2):168-75.

25. Weintraub WS, Harrison DG. C-reactive protein, inflammation and atherosclerosis: do we really understand it yet? Eur Heart J. 2000;21(12):958-60.

26. Pérez de Isla L, Alonso R, Mata N, Fernández-Pérez C, Muñiz O, Díaz-Díaz José L, et al. Predicting Cardiovascular Events in Familial Hypercholesterolemia. Circulation. 2017;135(22):2133-44.

27. Mirzaee S, Cameron JD. Familial hypercholesterolemia and cardiovascular risk stratification. Journal of clinical lipidology. 2018;12(5):1328-9.

TABLES

Table-1 Cohort basic characteristics

	Coronary Artery Disease	Absence of Coronary Artery Disease	P value
Sample	N= 40	N=20	*P* value
Males, n	33 (82%)	4 (20%)	<0.001
Type 2 DM, n	8 (20%)	3 (15%)	0.74
Hypertension, n	22 (55%)	8 (40%)	0.27
Smoker/Ex-Smoker	23 (58%)	10 (50%)	0.06
Previous CVA	3 (8%)	0 (0%)	0.46
Peripheral Vascular Disease	1 (3%)	0 (0%)	>0.99
Statin prescribed	14 (37%)	3 (15%)	0.08
LDL-C mmol/L, median	3.3	5.3	<0.001

CVA = Cerebrovascular accident; DM: Diabetes Mellitus, LDL-C = Low density lipoprotein cholesterol

Table 2: PCAT and EAT attenuation index in familial hypercholesterolemia patient cohorts

	Coronary Artery Disease	Absence of Coronary Artery Disease	*P* value
PCAT Attenuation Index (Global)	-85.3 HU ± 11.9	-90.6 HU ± 7.6	0.09
PCAT Attenuation Index (Per lesion analysis)	-80.9 HU ± 12.2	-90.6 HU ± 7.6	0.004*
EAT Attenuation	87.7 HU +/- 6.2	86.9 HU +/- 7.1	0.10

* Comparing per lesion attenuation in the CAD cohort with global PCAT attenuation in the absence of CAD cohort. This is because per lesion analysis is not possible in the absence of CAD.

EAT = Epicardial adipose tissue; HU = Hounsfield unit; PCAT = pericoronary adipose tissue

FIGURES

Figure 1: RCA segment plaque analysis with and without the presence of CAD

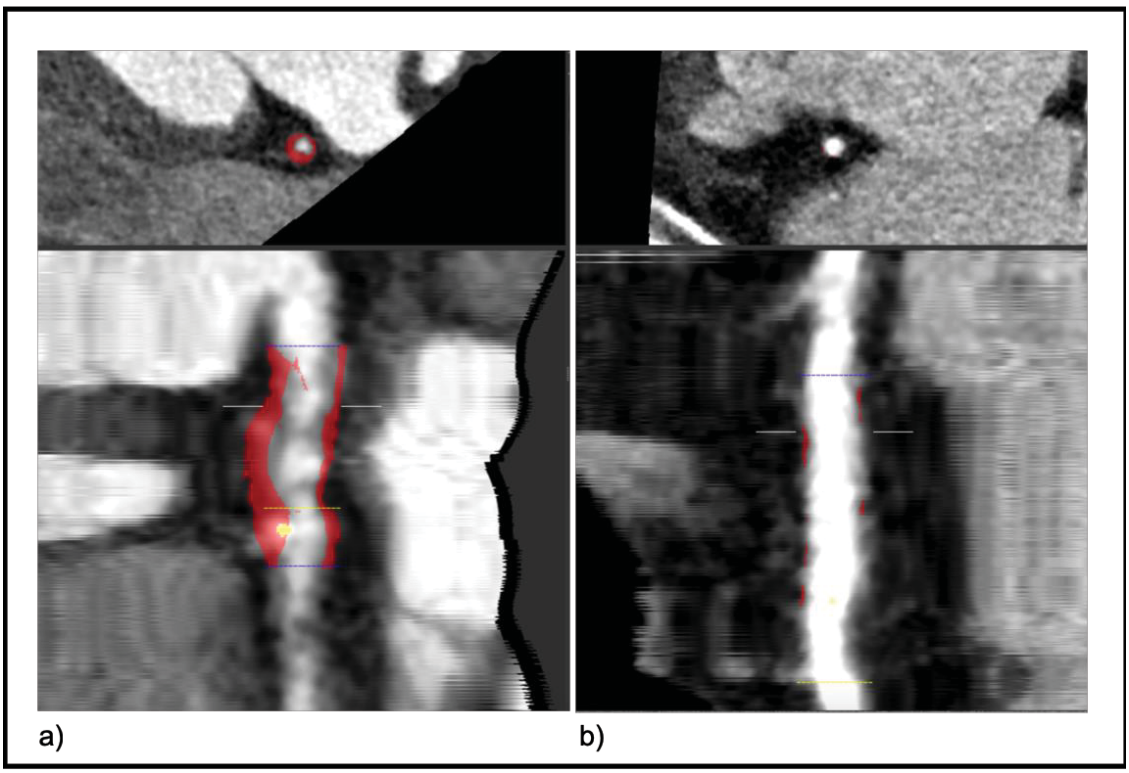
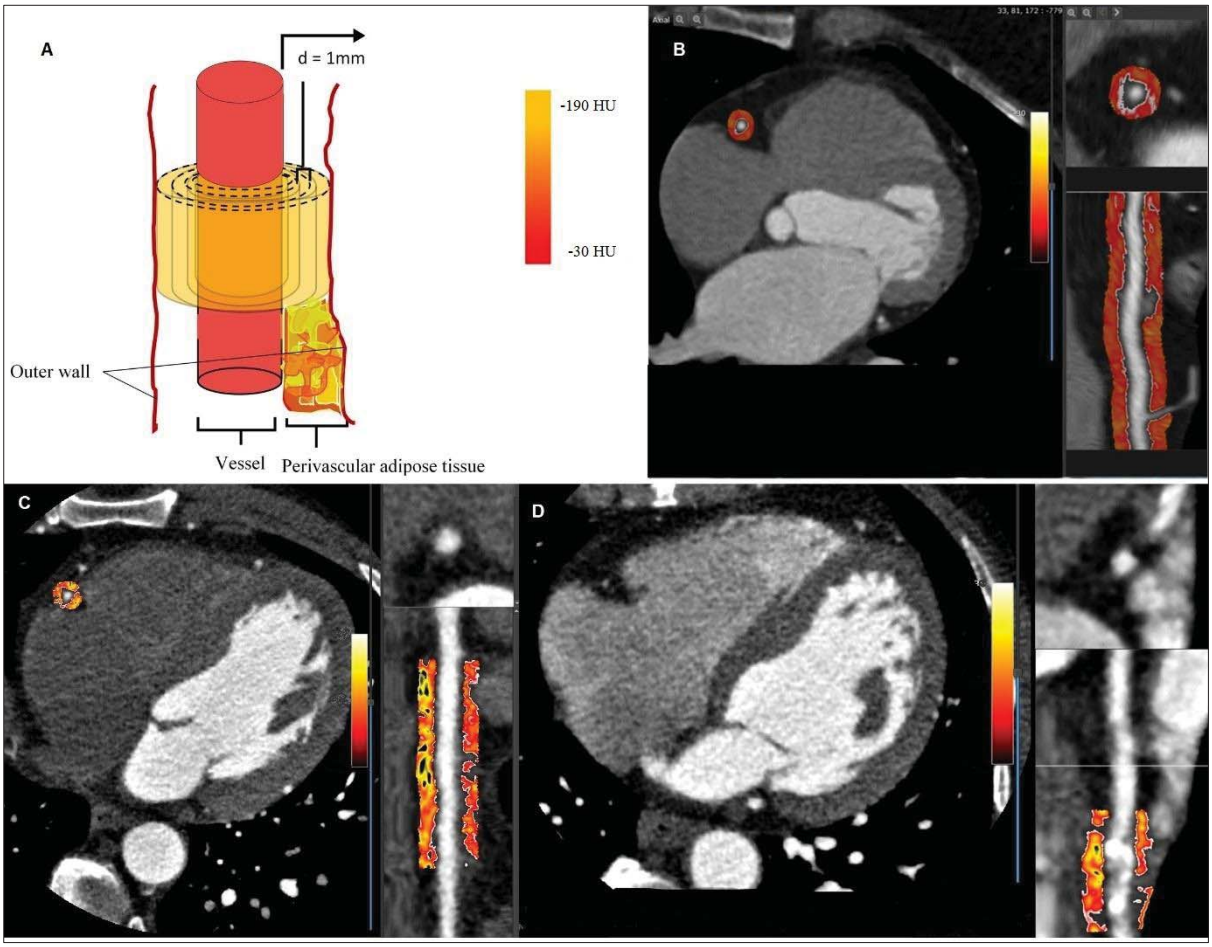


Figure 1 shows examples of plaque analysis completed utilizing AutoPlaque. A) shows the analysis of a proximal RCA in an individual with CAD. B) Shows plaque analysis of the RCA in a patient without CAD.

PCAT: Peri-Coronary Adipose Tissue; CAD: Coronary Artery Disease; RCA: Right coronary artery.

Figure 2: PCAT attenuation diagram (2-A) – Proximal RCA segment PCAT analysis in a patient with (2-B) and without (2-C) the presence of CAD- PCAT analysis in segmental LAD disease (2-D).



PCAT: Peri-Coronary Adipose Tissue; CAD: Coronary Artery Disease; HU: Hounsfield Unit; LAD: Left anterior descending RCA: Right coronary artery.

Figure 3: An example of EAT measurement utilizing QFAT

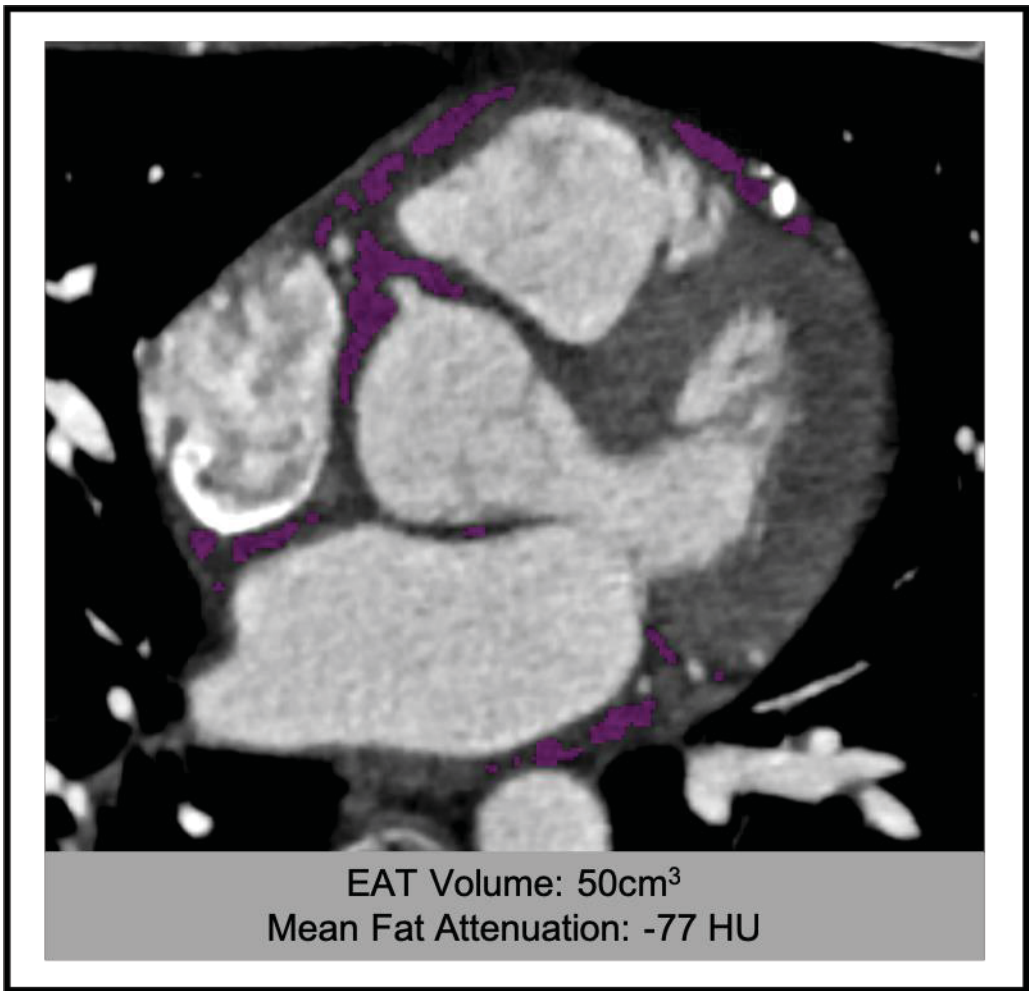


Figure 3 depicts epicardial fat analysis utilizing QFAT on CTCA. The epicardial fat is highlighted in purple in the above image.

Chapter 5

Tailoring a Novel Cardiometabolic Risk Stratification in Patients with Familial Hypercholesterolemia by Adding Oral Fat Tolerance Test

Tailoring a Novel Cardiometabolic Risk Stratification in Patients with Familial Hypercholesterolemia by Adding Oral Fat Tolerance Test

Sam Mirzaee[1], James CG Doery[2],Arthur Nasis[1], James D Cameron[1]

[1] Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia,

[2] Monash Health Pathology, Monash Health, Monash University, Melbourne, Australia

Corresponding Author:

Dr Sam Mirzaee

Monash Cardiovascular Research Centre, MonashHeart, Monash Health, Victoria, Australia

Email: sam.mirzaee@monash.edu

Tel: +61 3 9695 6666

Fax: +61 3 9594 4246

ABBREVIATIONS

ASCVD	Atherosclerotic cardiovascular disease
CAD	Coronary artery disease
CTCA	CT Coronary Angiogram
HeFH	Heterozygous Familial Hypercholesterolemia
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
OFTT	Oral fat tolerance test
PPL	Postprandial Lipaemia
RC	Remnant cholesterol
TG	Triglyceride

ABSTRACT

Background: Impaired postprandial remnant cholesterol (RC) metabolism results in high triglyceride (TG) in plasma and may intensify the risk of atherosclerotic cardiovascular disease (ASCVD) in familial hypercholesterolemia (FH). However, routine assessment of postprandial lipemia in FH has not been previously investigated.

Objective: We aimed to assess postprandial lipemia and chylomicron metabolism by implicating a simplified oral fat tolerance test (OFTT).

Methods: Thirty consecutive subjects with phenotypical FH (N_1=20 with ASCVD and N_2=10 without ASCVD) prospectively underwent a simplified OFTT (0,4h) using a dietary supplement containing 75.0 g monosaturated fat, 41.9 g carbohydrate and 7.2 g protein. FH was diagnosed using the Dutch Lipid Clinic Network criteria. Total cholesterol, TG and high-density lipoprotein (HDL)-cholesterol were measured with enzymatic, colorimetric assays on a Beckman Coulter AU 5800 analyser. RC was calculated as (Total cholesterol-LDL cholesterol-HDL cholesterol).

Results: Delta plasma TG for cases [mean age 49.9 ±11.6yr, median LDL 2.3 (1.7-4.4), median TG 1.2 (0.95-1.40) mmol/L], and 10 FH patients without ASCVD [mean age 46.4 ±13.8 yr, LDL 3.5 (2.4-4.5), TG 1.0 (0.60-1.30)] was measured. In cases with ASCVD, there was significant increased postprandial delta TG [1.50 (0.75-2.0) vs 0.35 (0.18-1.56), *p*= 0.01] and RC [1.2 (0.85-1.55) vs 0.35 (0.18-1.56), *p*= 0.01]. Postprandial dyslipidaemia in FH with and without ASCVD was independent to conventional risk factors, lipoprotein (a) level [0.18 (0.06-1.14) vs 0.23 (0.15-0.40) *p*=0.54], body mass index [(27.3 (3.7) vs 24.4 (4.2), *p*=0.06], HbA1C [5.8 (0.4) vs 5.6 (0.8), *p*= 0.29] and insulin plasma level [(11.5 (6.3) vs 7.0 (2.4), *P*=0.06].

Conclusions: Our results suggest that postprandial lipemia is significantly higher in FH subjects with ASCVD. Further trial is required to evaluate implications for additional lipid lowering therapy for further reduction of ASCVD in this high risk group.

Key words; familial hypercholesterolemia, coronary artery disease, post prandial lipaemia, remnant cholesterol.

INTRODUCTION

Heterozygous Familial hypercholesterolemia (HeFH) is an autosomal dominant and relatively common hereditary lipid disorder causing premature cardiovascular disease (CVD). (1, 2) The prevalence of HeFH reported between 1:200-350 in general population (3) and several published guidelines have focused on early detection and treatment of HeFH to reduce the further risk of development of CVD. (4-6) The typical form of HeFH is low density lipoprotein cholesterol (LDL-C) receptor defect, leading to elevated plasma LDL-C due to decreased hepato-cellular uptake of LDL-C. (7) Although it has been shown there is a positive correlation between LDL-C in plasma and the incidence of CVD (4), there is a significant heterogeneity in the incidence of CVD among patients with HeFH, even among those with identical genetic defect and plasma LDL-C concentration.

Therefore, the pathophysiological mechanism of development of CVD in patients with HeFH appears more complex than simply elevated LDL-C. Some of the known residual cardiovascular risk factors include hypertension, diabetes, smoking, obesity, postprandial (non-fasting) lipaemia and elevated lipoprotein (a) [Lp(a)]. (8, 9) There is no general consensus regarding definition and precise measurement of postprandial lipaemia (PPL), however in large body of literatures PPL mainly refers to the accumulation of plasma TG-rich lipoprotein particles (chylomicron, very low-density lipoprotein (VLDL) and their remnants) about 6–10 h after a fatty meal. (10, 11) Current evidence suggests a significant amount of chylomicron remnants clearance would be accomplished thru LDL receptors, therefore we expect patients with HeFH also have significant impaired post prandial lipemia. Even in HeFH with non-LDL receptor genetic defect significantly elevated LDL-C would compete with chylomicron remnant would result in impaired PPL. (11)

Current epidemiological evidence shows PPL is an important independent risk factor for CVD acting by several mechanisms including aggravating systemic inflammation, platelet aggregation, coagulopathy, and macrophage foam cell formation. (9) Non-fasting TG measurement is a simple

approach to evaluate PPL according to the recent European lipid guideline (12) , but it may not allow for a complete functional assessment of postprandial lipid metabolisms and potential abnormalities in high-risk group. We hypothesised postprandial lipid disturbance could be an additional metabolic defect and subsequently an atherogenic risk factor in patients with HeFH. Also, we proposed measurement of the non-fasting TG level may not be sufficient for evaluating CVD risks in patients with HeFH due to the described multifaceted pathophysiological mechanism, thus precise analysis of postprandial lipoprotein metabolism via a standard oral fat tolerance test (OFTT) is imperative.

METHODS

Study Population

We conducted a prospective, single-centre, non-randomised case-control study evaluating acute postprandial lipaemic response in total of 30 patients (N_1=20 with CVD and N_2=10 without CVD) with probable/definite FH using a Dutch Lipid Clinic Network Criteria (DLCNC) and ≥18 years of age from September 2018 to January 2019 at MonashHeart (Monash Health, Melbourne, Australia). We screened 48 FH patients attended outpatients lipid clinic, those had non-fasting TG < 2.0 mmol/L, DLCNC >5 and computed tomography coronary angiogram (CTCA) within the last 12 months were selected and subsequently we obtained written informed consents from all participants **(Figure-1).** Baseline height, weight, vital signs and relevant biochemical tests including thyroid function test (TFT), plasma insulin level, hemoglobulin A1C (HBA1-C) were measured. Basic characteristics and conventional cardiovascular risk factors, including hypertension, diabetes, smoking and medications were obtained from the hospital electronic medical records **(Table-1**). The study was approved by the Human Research and Ethics Committee of Monash Health (HREC/17/MonH/567).

Simplified Oral Fat Tolerance Test

Participants fasted overnight (>12 h) and attended the day care centre in the following morning. Baseline biochemistry and measurement were taken on the study visit. A dietary supplement was provided by the research team containing 75.0 g total fat (rapeseed and sunflower oil), 41.9 g carbohydrate and 7.2 g protein and flavoured with chocolate syrup consistent with previously validated simplified 4-h OFTT **(Table-2).** (12-14) Blood samples were taken twice at 0-4 h time point after the OFTT dietary supplement was ingested. We applied a pragmatic approach to study PPL by using a simplified OFTT in 4 hour post fat loading as the peak time of remnant lipoproteins level in plasma. (15) Participant remained fasted in the treatment room while awaiting second lipid profile testing. In terms of lipid lowering medications, fenofibrate and fish oil were discontinued at least 4 weeks before the study, however statin, evelocumab and ezetimibe were not interrupted.

Safety Monitoring

Since fat loading by OFTT may cause gastrointestinal symptoms such as nausea, vomiting, and diarrhea, we followed up the participant after 48 hours. We checked for all unfavourable symptoms related or unrelated to this study.

Assessment of coronary artery disease

All participants had undergone a clinically indicated CTCA on a 320-detector-row scanner (Aquilion Vision, Toshiba, Japan) at MonashHeart within the last 12 months. The image acquisitions were performed according to the departmental protocol (16) and established guidelines. (17) All studies were previously reported by two expert readers unaware of prospect of FH. The presence of significant CAD was defined as the presence of a lesion visually graded as having stenosis ≥50% of luminal diameter in a vessel of greater than 2mm diameter.

Laboratory Analyses

Lipid profile including total cholesterol, triglyceride and high-density lipoprotein cholesterol (HDL-C) were performed with enzymatic, colorimetric assays on a Beckman Coulter AU 5800 analyser (Beckman Coulter, Brea, CA, USA). The inter- and intra-assay percent coefficients of variation (%CVs) of these assays were <5 %. LDL-cholesterol was calculated according to the Friedewald equation. (18) The cut-off for abnormal postprandial TG levels was defined as >2 mmol/L. (12)

Characterization of phenotypical familial hypercholesterolemia

We used DLCNC for diagnosis of phenotypical FH defined as the numerical sum to each of the criteria: definite, score >8; probable, score 6–8; possible, score 3–5; and unlikely, score <3. (5) Simplified DLCNC include: personal history of early onset CAD (at age <55 years for men and <60 years for women (2 points), personal history of stroke (1 point); premature family history of CAD (1 point); physical examination, tendon xanthoma (6 points), arcus cornealis (4 points) and plasma LDL cholesterol >8.5 mmol/L (8 points), LDL cholesterol 6.5–8.4 (5 points), LDL cholesterol 5–6.4 mmol/L (3 points), LDL cholesterol 4–4.9 mmol/L (1 point). In case of being on lipid lowering therapy, plasma LDL-C level was corrected according to the potency and dose of specific statins or ezetimibe to estimate pre-treatment levels (19). In this study, phenotypic FH was defined as a score >5 (probable/definite FH).

Outcome measures

The primary outcome for this study was assessment of postprandial TG and TG rich lipoproteins (non-HDL lipoproteins) following the 4-h OFTT.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median and interquartile range in the case of non-normal distribution. Categorical variables are provided as frequencies

(percentages). The Shapiro-Wilk test was used to assess normality for continuous data. The Chi square or Fisher exact test was used for comparison of categorical variables. Continuous variables were compared with Student's *t* test and the Mann-Whitney U test according to normality. Statistical significance was defined as $p < 0.05$ (two-tailed). PCAT measurements were indexed by time between scans. Statistical analyses was performed with STATA-14 (StataCorp, College Station, TX, USA).

RESULTS

All thirty patients with probable/definite phenotypical FH voluntarily ingested high fat dietary supplements within 15 minutes, followed by 4 hours blood test and observation in the treatment room. There was no significant difference in basic characteristics between two groups, however majority of patients with early onset of CAD were male.

Plasma Postprandial Lipemic changes in response to OFTT

The peak postprandial TG would be 3-4 hours after diet, therefore we performed the second lipid profile after 4 hours of commencing supplement. As demonstrated in **Table-3,** there was a significant elevation in median TG (*p* 0.013) and non-HDL cholesterol (*p* 0.017) in plasma level followed by OFTT between two groups.

Potential contributing factors in response to OFTT

It was also observed at 0 hour there was no meaningful difference between potential contributing factors including diabetes, insulin level, BMI and statin use which may result in PPL **(Table-1)**.

DISCUSSION

OFTT has been broadly used in human research to assess PPL and further risk stratification, but there was no consensus on lipid guidelines for clinical utility and procedures. We adopted a protocol

based on recommendations from an expert panel statement using simplified OFTT measuring TG 4 hours after ingesting moderate amount of fat (30-70gr). (20) The results from our study demonstrate that despite of having similar fasting TG and remnant cholesterols in FH patients with and without significant ASCVD, those with significant disease have a clinically meaningful changes in TG and remnant cholesterol lipoproteins during OFTT. Secondary findings were this significant hyperlipidaemia in response to fat loading in OFTT was independent to the other potential contributing factors such as insulin level, HbA1-C and BMI. Postprandial lipaemia and elevated residual remnant cholesterol in patients with FH and also high risk patients with ASCVD have previously demonstrated (20, 21); however, the role of OFTT in risk assessment among patient with FH had not been reported.

To the best of our knowledge, the role of OFTT in assessment of residual cardiovascular risk among FH patients has not been previously investigated. Our results are keeping with the literature, postprandial lipaemia and higher remnant cholesterol are independently correlated with atherogenesis in high risk population. (22-24) We identified that simplified OFTT will unmask a subclinical postprandial lipaemia in high risk patients with FH who had an unremarkable random TG, and have also been receiving statin therapy. This can also be considered as an additional risk stratification tool and further intensive treatment by aggressive lifestyle change and pharmacotherapy. Further interventional trials evaluating combined lipid lowering regiment (eg. n-3 fatty acids eicosapentaenoic acid (EPA) and fibrate) in this high-risk group is also an unmet need.

The other strength of our trial include simplicity, practicality in conjunction with standardisation of PPL evaluation. Since it is paramount to assess postprandial TG response with a standard high-fat loading and controlled interfering variables, we tried to minimise inconsistency by prolonged fasting, controlling the amount of energy consumption and fluid intake during the OFTT. This result

highlights that biochemical and physiological response to a diet can be complicated, and only a random non-fasting lipid profile may not been sufficient to reveal PPL at least in the FH setting.

LIMITATION

We did not perform genetic testings to genetically confirm or differentiate various types of genetic defects, and apolipoprotein-E genotypes as a potential contributing factor on PPL in our study population.(25) Postprandial glucose and insulin level in plasma was not tested; however, there is a poor agreement in peak concentration of insulin during OFTT. (26) PPL has a complex pathophysiological mechanism and some unknown factors impacting on fatty acid oxidation in the liver and triglyceride removal from plasma into adipose and muscle tissue may have not been addressed in this study. (27) Finally, we cannot entirely exclude confounding effects of the evening meal and physical activities performed within 24 hours prior to the OFTT.

CONCLUSION

This study suggests that postprandial lipemia is significantly higher in FH subjects on stating therapy with ASCVD utilising simplified OFTT (0-4h). Further trial is required to evaluate implications for aggressive lifestyle modification with additional TG lowering therapy for further reduction of ASCVD in this high-risk group.

Disclosure

Dr Mirzaee has received consultancy fees from Amgen, Sanofi and Boehringer Ingelheim. The other authors have no conflicts of interest to disclose.

References

1. Hovingh GK, Davidson MH, Kastelein JJP, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. European Heart Journal. 2013;34(13):962-71.

2. Watts GF, Shaw JE, Pang J, Magliano DJ, Jennings GL, Carrington MJ. Prevalence and treatment of familial hypercholesterolaemia in Australian communities. International journal of cardiology. 2015;185:69-71.

3. Bell DA, Watts GF. Progress in the care of familial hypercholesterolaemia: 2016. The Medical journal of Australia. 2016;205(5):232-6.

4. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013;34(45):3478-90a.

5. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. International journal of cardiology. 2014;171(3):309-25.

6. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36(36):2425-37.

7. Goldstein JL, Brown MS. The LDL receptor. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(4):431-8.

8. Chan DC, Watts GF. Postprandial lipoprotein metabolism in familial hypercholesterolemia: thinking outside the box. Metabolism: clinical and experimental. 2012;61(1):3-11.

9. Masuda D, Yamashita S. Postprandial Hyperlipidemia and Remnant Lipoproteins. Journal of atherosclerosis and thrombosis. 2017;24(2):95-109.

10. Genovefa DK, Dimitri PM, Borge GN, Helen B, George P. Definition of Postprandial Lipaemia. Current vascular pharmacology. 2011;9(3):292-301.

11. Watts GF. Postprandial lipaemia in familial hypercholesterolaemia: clinical and metabolic significance. Atherosclerosis. 2000;148(2):426-8.

12. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944-58.

13. Maraki M, Aggelopoulou N, Christodoulou N, Katsarou C, Anapliotis P, Kavouras SA, et al. Validity of abbreviated oral fat tolerance tests for assessing postprandial lipemia. Clinical Nutrition. 2011;30(6):852-7.

14. Weiss EP, Fields DA, Mittendorfer B, Haverkort MAD, Klein S. Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test. Metabolism - Clinical and Experimental. 2008;57(10):1479-85.

15. Mihas C, Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, et al. Diagnostic value of postprandial triglyceride testing in healthy subjects: a meta-analysis. Current vascular pharmacology. 2011;9(3):271-80.

16. Wong DT, Soh SY, Ko BS, Cameron JD, Crossett M, Nasis A, et al. Superior CT coronary angiography image quality at lower radiation exposure with second generation 320-detector row CT in patients with elevated heart rate: a comparison with first generation 320-detector row CT. Cardiovascular diagnosis and therapy. 2014;4(4):299-306.

17. Abbara S, Arbab-Zadeh A, Callister TQ, Desai MY, Mamuya W, Thomson L, et al. SCCT guidelines for performance of coronary computed tomographic angiography: A report of the Society of Cardiovascular Computed Tomography Guidelines Committee. Journal of Cardiovascular Computed Tomography. 2009;3(3):190-204.

18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502.

19. Haralambos K, Whatley SD, Edwards R, Gingell R, Townsend D, Ashfield-Watt P, et al. Clinical experience of scoring criteria for Familial Hypercholesterolaemia (FH) genetic testing in Wales. Atherosclerosis. 2015;240(1):190-6.

20. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, et al. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. Current vascular pharmacology. 2011;9(3):258-70.

21. Perez-Martinez P, Alcala-Diaz JF, Kabagambe EK, Garcia-Rios A, Tsai MY, Delgado-Lista J, et al. Assessment of postprandial triglycerides in clinical practice: Validation in a general population and coronary heart disease patients. Journal of clinical lipidology. 2016;10(5):1163-71.

22. Jackson KG, Poppitt SD, Minihane AM. Postprandial lipemia and cardiovascular disease risk: Interrelationships between dietary, physiological and genetic determinants. Atherosclerosis. 2012;220(1):22-33.

23. Borén J, Matikainen N, Adiels M, Taskinen M-R. Postprandial hypertriglyceridemia as a coronary risk factor. Clinica Chimica Acta. 2014;431:131-42.

24. Hyson D, Rutledge JC, Berglund L. Postprandial lipemia and cardiovascular disease. Curr Atheroscler Rep. 2003;5(6):437-44.

25. Tácito LHB, Yamada LN, de Souza Pinhel MA, Yugar-Toledo JC, Souza DRS. Influence of Apolipoprotein E on the Lipid Profile and Postprandial Triglyceride Levels in Brazilian Postmenopausal Women With Artery Disease. Clin Med Insights Cardiol. 2017;11:1179546817731110-.

26. O'Doherty AF, Sathyapalan T, Rigby AS, Ingle L, Carroll S. The repeatability of the abbreviated (4-h) Oral Fat Tolerance Test and influence of prior acute aerobic exercise. European journal of nutrition. 2018;57(1):309-18.

27. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. Diabetes Research and Clinical Practice. 2011;93:S52-S9.

Table-1

Baseline characteristics of participants

	Cases (n= 20)	Controls (n=10)	*p* Value
Age , years (SD)	49.9 (11.6)	46.4 (13.8)	0.476
Gender, Male n (%)	16 (80)	1 (10)	<0.001
BMI (kg/m^2)	27.3 (3.7)	24.4 (4.2)	0.067
Blood Pressure (pre)			
Systolic, mmHg (SD)	117.1 (11.1)	125.4 (22.5)	0.183
Diastolic	71.3 (7.7)	73.8 (9.5)	0.444
Blood Pressure (post)			
Systolic, mmHg (SD)	116.0 (11.6)	123.8 (16.6)	0.118
Diastolic	67.6 (9.1)	70.8 (10.5)	0.172
Heart Rate			
Pre	62.6 (9.6)	71.5 (11.6)	0.033
Post	65.1 (8.0)	76.4 (14.8)	0.011
Hypertension, n (%)	10 (50)	2 (20.0)	0.114
Diabetes, n (%)	1 (5.0)	0	0.472
Smoking, n (%)			
Active	2 (10)	0	0.301
Ex-smoker	10 (50)	2 (20)	0.114
DLCNC Score, n (SD)	10.9 (4.7)	10.9 (4.3)	1.0
Arcus Cornealis, n (%)	8 (40)	4 (40)	1.0
AT Xanthoma	9 (45)	7 (70)	0.196
Baseline Lp (a) g/L *	0.18 (0.06-1.14)	0.23 (0.15-0.40)	0.546
TSH	1.84 (0.94)	1.54 (0.92)	0.431
Insulin mu/L	11.5 (6.3)	7.0 (2.4)	0.063
HbA1c (%)	5.8 (0.4)	5.6 (0.8)	0.297
Angiographic Significant CAD	20 (100)	0	<0.001
Aspirin, n (%)	17 (85.0)	2 (20)	<0.001
Statin, n (%)	16 (80.0)	6 (60)	0.243
Ezetimibe, n (%)	8 (40.0)	4 (40.0)	1.0
Beta-blocker, n (%)	11 (55.0)	1 (10.0)	0.018
ACEi/ARB, n (%)	13 (65.0)	1 (10.0)	0.004
Evolucumab, n (%)	3 (15.0)	0	0.197

ACEi: Angiotensin convertase inhibitors; ARB: Angiotensin II receptor blockers; BMI: body mass index; CAD: coronary artery disease; DLCNC: Dutch Lipid Clinical Network Criteria. *Median (Q1, Q3)

Table-2

Oral Fat Tolerance Test dietary supplement and other composition

Volume (ml)	**150**
Energy (kJ)	2775.0
Total Fat (g)	75.0
Saturated fat (g)	7.9
Carbohydrate (g)	41.9
Protein (g)	7.2
Fibre (g)	2.0

Table-3

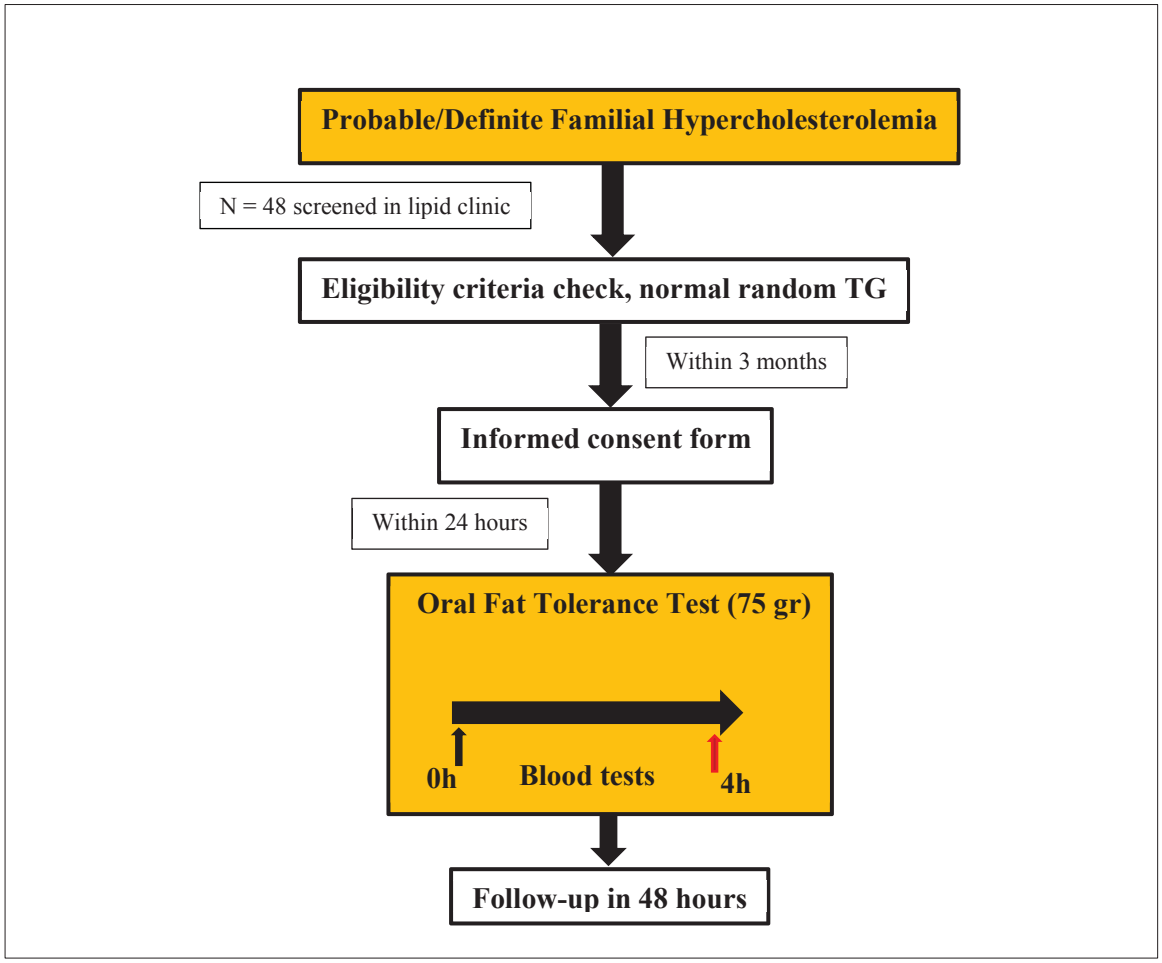
Comparing the result of oral fat tolerance test between two groups.

	Cases (n=20)	**Controls (n=10)**	***p* value**
Baseline Lipids (0h)*			
Total Cholesterol (mmol/L)	4.1 (3.30-6.30)	5.20 (4.9-6.2)	0.194
Total Triglycerides (mmol/L)	1.2 (0.95-1.40)	1.0 (0.60-1.30)	0.081
LDL-Cholesterol (mmol/L)	2.3 (1.7-4.4)	3.5 (2.4-4.5)	0.281
HDL-Cholesterol (mmol/L)	1.3 (1.1-1.5)	1.5 (1.1-2.2)	0.163
Remnant Cholesterol (mmol/L)	0.50 (0.40-0.60)	0.50 (0.30-0.60)	0.198
Post Fat Challenge Test (4h)*			
Total Cholesterol (mmol/L)	4.1 (3.4-5.9)	5.1 (4.9-6.2)	0.165
Total Triglycerides (mmol/L)	2.9 (2.0-3.3)	1.3 (0.90-1.7)	***0.025***
LDL-Cholesterol (mmol/L)	1.30 (0.95-3.80)	3.5 (2.1-4.4)	0.085
HDL-Cholesterol (mmol/L)	1.2 (1.0-1.45)	1.50 (1.1-2.2)	0.143
Remnant Cholesterol (mmol/L)	1.2 (0.85-1.55)	0.50 (0.40-0.80)	***0.017***
Delta Triglycerides (mmol/L), (Post-Pre)	1.50 (0.75-2.0)	0.35 (0.18-1.56)	***0.013***

*Median (Q1-Q3)

Figure-1

Patient selection and study design.



TG: triglyceride

CHAPTER 6:

Conclusion and Future Direction

Conclusion and Future Direction

The result of this theses have advanced the understanding about high risk individuals with phenotypical FH and premature CAD in four key areas.

Firstly the results presented specify the prevalence of FH and their prognoses among patients with premature CAD admitted in CCU. The frequency rate of FH in our network was relatively common among patients with ACS (11.2%), similar to the findings in current literature [(1)] and the vast majority of FH patients were underdiagnosed and suboptimally treated. In this subgroup population based retrospective study, we found an independent association between FH, higher plasma LDL-C level and incidence of fatal ventricular arrhythmia complicating AMI. At present, there remains insufficient information to support the exact pathophysiological mechanism of higher mortality and poor prognosis in FH, however our study suggests that the mortality associated with AMI in patients with FH may be due to non-arrhythmic complications.

Secondly this is the first report of evaluation of knowledge of awareness about FH in hospital based healthcare providers involved in the management of ACS. Current data suggesting substantial deficit in the knowledge of FH among primary care physicians. [(2)] Our multicentre survey demonstrated despite of high frequency rate of FH in CCU, there was a suboptimal awareness and proficiency among hospital based healthcare providers involved in the management of ACS.

Thirdly the results presented demonstrate that non-invasive assessment of vascular inflammation using PCAT provided additional information about the risk of developing cardiac disease in patients with FH. Furthermore, higher PCAT attenuation in patients with CAD compared to those without CAD, perhaps reflecting a greater degree of coronary inflammation. These observations are required to advance knowledge about heterogeneity among FH patients but also contribute in development of specific risk stratification tool in FH and future treatment. New international guidelines recommending cardiovascular risk assessment tools used for general population should not be used in high risk groups such as FH. [(3)]

Fourthly this is the first report of assessment of postprandial lipemia and chylomicron metabolism by implicating a simplified oral fat tolerance test (OFTT 0h-4h) in FH patients. Current evidence suggests TG rich lipoproteins and post parandial lipaemia is a residual risk factor for development of CAD. [4] We also know a significant amount of chylomicron remnants clearance would be accomplished thru LDL receptors, therefore we hypothesised patients with HeFH and CVD also have significant impaired post prandial lipemia.[4] Our data suggests that postprandial lipemia is substantially higher in FH patients with CVD compared with those without CVD. This indicate the necessity of additional dynamic tests to unmask post prandial lipaemia. Post prandial lipaemia may explain the mechanism of progressive atherogeneis among patients who have been receiving treatment for LDL-C with reasonably controlled lipid profile. This information can also be extended to the care of all patients with high risk cardiovascular disease to improve their clinical outcome.

Education and a holistic approach towards FH patients through an integrated model of care will improve knowledge and awareness of health care professional and subsequently may enhance the current standard of care for FH patients in coronary care.

Future prospective multicentre trials will undoubtedly be required to further validate novel parameters using cardiac CT to assess vascular inflammation and qualitative plaque analysis. These will be important to better understand the development of CAD and further treatment not only in FH patients, also in all high risk populations.

Additionally, future prospective multicentre studies will be required to evaluate implications for aggressive lifestyle modification with additional TG lowering therapy for further reduction of cardiovascular events in association with post prandial lipaemia among individuals with FH or high risk for cardiovascular disease.

Furthermore the results of this theses support the concept of patients with FH are at high risk of developing CVD due to lipid metabolism issue, and requiring a multifaceted approach and an integrated model of care to enhance FH detection and their current standard of care.

References

1- Pang J, Sullivan DR, Harada-Shiba M, Ding PY, Selvey S, Ali S, et al. Significant gaps in awareness of familial hypercholesterolemia among physicians in selected Asia-Pacific countries: a pilot study. Journal of clinical lipidology. 2015;9(1):42-8.

2- Bell DA, Garton-Smith J, Vickery A, Kirke AB, Pang J, Bates TR, et al. Familial Hypercholesterolaemia in Primary Care: Knowledge and Practices among General Practitioners in Western Australia. Heart, Lung and Circulation. 2014;23(4):309-13

3- Mach F. et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). European Heart Journal. 2019.

4- Watts GF. Postprandial lipaemia in familial hypercholesterolaemia: clinical and metabolic significance. Atherosclerosis. 2000;148 (2):426-8.

Appendix

APPENDIX

PREAMBLE

Seven articles have been attached in the appendix.

The first is a “Brief Communication” article which describes different aetiologies for “PCSK 9 inhibitor non-response” in patients with FH. This article for the first time highlights a faulty injection can simply lead to suboptimal response to PCSK9 inhibitors and subsequently suggested a stepwise strategy in assessment of “PCSK 9 inhibitor non-response”.

The second article is another “Brief Communication” paper that describes a few aspects in care of patients with FH and coexisting reno-vascular disease: 1) Patients with premature atherosclerotic renal artery stenosis (male < 55 and female <60 years of age) should be screened for FH, 2) Early onset hypertension (<30 years of age) in FH patients should prompt clinicians to consider further investigation of reno-vascular disease, 3) FH is a systemic panvascular disease and a high index of clinical suspicion for subclinical atherosclerosis is needed in management of these high risk patients, 4) Early intense pharmacological in addition to diet and lifestyle modification is the key lipid lowering approach in patients with FH and other conventional risk factors such as hypertension to prevent further development of serious atherosclerotic complications.

The third include four letters which were sent and received in relation to two original research articles provided information about the cardiovascular outcome in patients with FH. In these two letters, we highlighted firstly cardiovascular risk stratification tools using in general population should not be used in patients with FH as they can erroneously misguide clinicians. Subsequently, we signified an unmet need for a dedicated novel cardiovascular risk stratification method for both primary and secondary prevention in FH patients. The second letter indicated that FH is

panvascular disease the importance of intensive lipid lowering treatment in this subgroup. We emphasised ramification of FH is not limited to coronary arteries and a normal cardiac CT should not discourage and clinicians to underestimate the future risk of atherosclerotic cardiovascular diseases.

The fifth article is a “review article” which looks over the impact of environmental factors and nutrition in the management of patients with FH. This article highlights variable clinical manifestations of FH in association with multiple causes including genetic and non-genetic elements. Once again this article provide information for clinicians and recommendations to acknowledge modifiable environmental factors impact on FH and to adopt a holistic approach in the management of this treatable disease.

The sixth paper is a review article which summarises the cardiovascular outcome of several clinical trials studying the role of additional high dose omega-3 fatty acids in cardiovascular prevention. In this paper, we highlight triglyceride levels may be one of the several modifiable biomarkers contributing in the residual cardiovascular risk in high risk patients, and further management with high-dose omega-3 fatty acids present a highly effective intervention. This data is particularly helpful in the future research and clinical management of residual cardiovascular disease in patients with FH as we demonstrated in Chapter 5 of this theses.

Finally the last article is a study protocol for the **P**rotein **O**mega-3 and Vitami**N**-**D** **E**xercise **R**esearch (PONDER), which I was involved with development and in advisory team. This is a fascinating first randomised controlled trial to study the combined effects of a multimodal exercise programme with omega-3 (900 mg EPA and 600 mg DHA), vitamin-D and protein supplementation on the primary end point of cognition in older adults with subjective memory impairment and secondary endpoints of cardiovascular function. This study is currently in the follow-up stage investigating designated

clinical outcomes. This data will be particularly important as it will illustrate the association of lifestyle and environmental factors in combination with omega-3 treatment on several clinical outcomes including cardiovascular function.

Heart, Lung and Circulation (2018) **28,** e21–e22
1443-9506/04/$36.00
https://doi.org/10.1016/j.hlc.2018.03.009

BRIEF COMMUNICATION

A Small Change Can Make a Big Difference: A Lesson from Evolocumab

Sam Mirzaee, MD, FRACP [*], Paul M. Thein, MBBS, Dennis Wong, MBBS, FRACP, Arthur Nasis, MD, FRACP

Monash Cardiovascular Research Centre, MonashHEART, Melbourne, Vic, Australia

Received 22 December 2017; received in revised form 13 February 2018; accepted 7 March 2018; online published-ahead-of-print 20 March 2018

Background Evolocumab is an expensive proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor which has been shown to significantly improve cardiovascular outcomes in high risk patients.

Methods This is a case study describing a stepwise approach to ''PCSK9 inhibitor non-response" in a patient with familial hypercholesterolaemia. There are a few described pathophysiological mechanisms for ''PCSK9 inhibitor non-response" including homozygous LDL-C receptor-negative mutations and alteration in the binding site of PCSK9 inhibitors.

Results We report the case of a 41-year-old woman with familial hypercholesterolaemia and premature cardiovascular disease, who was non-responsive to the action of PCSK9 inhibitor solely due to the incorrect subcutaneous injection technique.

Conclusions This case study highlights the importance of reviewing the accuracy of SC injection technique in patients with minimal or no response to PCSK9 inhibitors prior to proceeding to costly genetic testing.

Keywords Evolocumab • PCSK9 inhibitor • Familial hypercholesterolaemia • Coronary artery disease

Background

Familial hypercholesterolaemia (FH) is a common monogenic lipid disorder characterised by elevated plasma low-density lipoprotein cholesterol (LDL-C). Patients with FH are at significantly elevated risk for early onset cardiovascular events and death from coronary heart disease [1].

Evolocumab is a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor that binds to serum PCSK9 and slows down LDL-C receptor degradation resulting in lowering of circulating LDL-C [2]. Evolocumab is currently available in Australia on the Pharmaceutical Benefit Scheme for patients with FH diagnosed by the Dutch Lipid Clinical Network Criteria (DLCNC). The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial studied the cardiovascular outcomes in patients at high risk for cardiovascular disease taking evolocumab on a background of statin therapy and showed a significant improvement in cardiovascular events [3].

Evolocumab is an expensive medication, and the clinical cost effectiveness of PCSK9 inhibitors has been under scrutiny from a public health perspective. Several authors have suggested that the best approach to delivering the potential health benefits of PCSK9 inhibitors therapy at a reasonable cost is by reducing the price of the drug [4]. Furthermore, selection of high risk patients, adequate education and clinical vigilance should be at the cornerstone of PCSK9 inhibitor administration.

Formerly, a subgroup of patients with genetic mutations described as ''PCSK9 inhibitor non-responsive", including homozygous receptor negative mutations resulting in LDL-C receptor inactivity, and an alteration in the PCSK9 binding site, have been postulated as other mechanisms of non-responsiveness [5].

*Corresponding author: Monash Medical Centre, Monash Health, 246 Clayton Road, Clayton, Vic 3168, Australia. Tel.: +61 3 9594 6666. Email: Sam.Mirzaee@Monashhealth.org

Case

A 41-year-old woman was admitted to hospital with acute anterior ST elevation myocardial infarction in January 2017 when she was also diagnosed with FH. Her initial biochemistry tests revealed TC = 10.1, LDL-C = 8.2, TG = 1.9 and HDL = 1.1 mmol/L. She also reported a strong family history of premature coronary artery disease and elevated serum cholesterol in her first degree relatives. Her other cardiovascular risk factors included hypertension and smoking. Physical examination revealed bilateral arcus cornealis and Achilles tendon xanthoma confirming a phenotypic diagnosis of FH with DLCNC of 14. Her condition became complicated by ischaemic cardiomyopathy with reduced left ventricular ejection fraction of 35%, assessed by transthoracic echocardiogram. She received daily atorvastatin 80 mg and ezetimibe 10 mg as initial LDL-C lowering agents.

Two months later her LDL-C level had reduced to 5 mmol/L in conjunction with diet and lifestyle modification. Subsequently, she was commenced on evolocumab subcutaneous injections of 140 mg every fortnight. Eight weeks later, the LDL-C level was similar at 4.7 mmol/L, despite having received four injections of evolocumab.

In the first instance, the injection technique was reviewed and was found to be incorrect. Several errors were observed while she was demonstrating the technique by an injection simulator: firstly a ''stretch method'' was used on the abdominal site (instead of the preferred ''pinch method''); secondly, the selected region of skin comprised of hard consolidation (likely thickened subcutaneous fat); finally low pressure was applied over the skin and perhaps this led to either an intradermal injection or into an area with substantial thickened subcutaneous fat. As a result, there was minimal or no distribution of the medication into the subcutaneous tissue. The injection technique was rectified and evolocumab therapy was continued under the supervision of a medical practitioner. Six weeks later lipid profile showed a marked improvement with TC = 2.5, LDL-C = 1.0, TG = 1.0 and HDL = 1.1 mmol/L. This case highlights that a faulty injection technique due to intradermal rather than subcutaneous injection, as well as injecting to an area with thickened subcutaneous fat, resulted in suboptimal absorption and consequently ''PCSK9 inhibitor non response''. If not recognised, this could lead clinicians towards costly genetic testing to search for rare gene mutations.

Discussion

Evolocumab for subcutaneous administration is supplied in a single-use prefilled auto-injector containing 140 mg in 1 ml of solution. It is recommended that this should be injected by a well-trained individual until the patient becomes comfortable and skilled to perform self-injection. The injection should be administered in rotating sites over the abdomen (except for a two-inch area around navel), thigh and outer area of upper arm. The auto-injector should be held perpendicular (90°) to the stretched or pinched skin, depending on site of injection (''pinch method'' preferred for abdominal site and ''stretch method'' for thigh and upper arm). Subsequently, it needs to be firmly pushed onto the skin until it stops moving. Finally, the start button is pressed held for 15 seconds until injection is completed.

Furthermore, perfusion characteristics of the tissue, namely functional capillary density, is an important element in the effectiveness of subcutaneous medications [6]. Although there are only limited data about factors which may potentially interfere with delivering of evolocumab from subcutaneous tissue, the injection should be avoided into areas of skin which are hard, thickened, bruised, red, tender or over a tattoo as the histology of the region may have been altered [7].

Conclusion

PCSK9 inhibitors are an expensive but effective treatment for patients with FH at elevated cardiovascular risk. This case demonstrates that faulty injection technique is a potential cause of ''PCSK9 inhibitor non-response'' which can simply be managed by adequate education.

- Evolocumab, a PCSK9 inhibitor, is an effective lipid lowering agent in patients with familial hypercholesterolaemia
- Clinical vigilance for non-responsiveness to PCSK9 inhibitors is required
- A simple faulty injection technique can lead to PCSK9 inhibitor non-response
- Other causes of PCSK9 inhibitors non-response include genetic mutations and immunogenicity.

Conflict of Interest

SM has received honorarium and study grant from Amgen.

References

[1] Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: current perspectives on diagnosis and treatment. Atherosclerosis 2012;223(2):262–8.
[2] Giugliano RP, Pedersen TR, Park J-G, De Ferrari GM, Gaciong ZA, Ceska R, et al. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. Lancet 2017;390(10106):1962–71.
[3] Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 2017;376(18):1713–22.
[4] Kazi DS, Penko J, Coxson PG, Moran AE, Ollendorf DA, Tice JA, et al. Updated cost-effectiveness analysis of pcsk9 inhibitors based on the results of the fourier trial. JAMA 2017;318(8):748–50.
[5] Losh C, Underberg J. PCSK9 inhibitor non-response in a patient with preserved LDL receptor function: a case study. J Clin Lipidol 2016;10(3):719–20.
[6] Kim H, Park H, Lee SJ. Effective method for drug injection into subcutaneous tissue. Sci Rep 2017;7:9613.
[7] Shinohara MM. Complications of decorative tattoo. Clin Dermatol 2016;34(2):287–92.

Familial Hypercholesterolemia With Coexisting Renovascular Stenosis and Premature Coronary Artery Disease

Ojas H. Mehta, James D. Cameron, and Sam Mirzaee

Familial hypercholesterolemia (FH) is a common hereditary lipid disorder associated with substantial risk of premature atherosclerotic cardiovascular disease. We report an interesting newly diagnosed index case of FH in a 31-year-old man who presented to the hospital with an ST-elevated myocardial infarction. He had a background of inadequately treated hypertension and hypercholesterolemia. Further investigations raised the possibility of secondary hypertension after the identification of renal artery stenosis, in addition to other areas of mesenteric arterial stenoses. Our patient's case highlights that early-onset hypertension and hypercholesterolemia in a young individual may be an early manifestation of FH requiring high clinical vigilance and awareness.

Keywords: atherosclerosis; blood pressure; coronary artery disease; familial hypercholesterolemia; hypertension; renal artery stenosis

doi:10.1093/ajh/hpz037

Familial hypercholesterolemia (FH) is a disorder of low-density lipoprotein cholesterol (LDL-C) metabolism and has an autosomal dominant pattern of inheritance. FH is strongly associated with early-onset atherosclerotic cardiovascular disease (ASCVD) including coronary artery disease, stroke, and peripheral arterial disease. Other clinical manifestations of FH include severely elevated serum LDL-C levels, tendon xanthomas, arcus cornealis before 45 years of age, a family history of high LDL-C, and early-onset ASCVD in a first-degree relative.[1]

Current evidence shows that despite guidelines for FH screening, FH is significantly underdiagnosed and the prevalence of FH is higher than formerly expected, approximately in the range of 1 in 200–300.[2,3] Often, the late diagnosis of FH follows a major coronary artery event. Among the most widely used FH clinical criteria, the Dutch Lipid Clinical Network scoring system has the highest sensitivity in detecting FH.[4] Mutations in the 3 main genes implicated in FH (LDL-receptor gene, apolipoprotein B, and proprotein convertase subtilisin/kexin type 9) account for approximately only 40% patients with phenotypical FH.[5] Thus, despite controversy in different methods of assessment, the diagnosis of FH is often made clinically.[5,6]

CASE PRESENTATION

A 31-year-old man of Anglo-Indian descent was admitted to our coronary care unit with inferior ST-elevated myocardial infarction acute coronary syndrome complicated by moderate ischemic cardiomyopathy. He was treated with primary percutaneous intervention and stenting followed by cardioprotective medications. His past medical history included suboptimally treated hypertension diagnosed 3 years earlier on ambulatory blood pressure (BP) monitoring. He has had poorly controlled BP with systolic readings up to 230–240 mm Hg. He was also diagnosed with hypercholesterolemia 2 years earlier with a fasting total cholesterol level of 9.7 mmol/l (375 mg/dl) and LDL-C of 7.0 (271 mmol/l). He had received intermittent medical therapy with amlodipine for his hypertension and a statin for hypercholesterolemia but self-ceased these in favor of treatment *via* only intense lifestyle modification. His physical activity included intensive exercise for at least 1 hour with 5 sessions per week. In addition, our patient had a strong family history of hypercholesterolemia and premature ASCVD, with 2 uncles having coronary artery disease at young ages and eventually dying from myocardial infarctions at ages 52 and 59. Three paternal uncles and one paternal aunt had hypercholesterolemia.

On physical examination, body habitus was normal with body mass index 25 kg/m^2 and waist circumference 76 cm. Even though digital extensor tendon xanthomata were absent, he had minor bilateral thickening of Achilles tendons consistent with tendon xanthoma, left patellar tendon xanthoma, and bilateral arcus cornealis (Figure 1). Laboratory examination revealed substantially elevated serum concentrations of total cholesterol 8.3 mmol/l (321 mg/dl), LDL-C 6.2 mmol/l (240 mg/dl), high-density lipoprotein cholesterol 1.3 mmol/l (50 mg/dl), triglyceride 1.8 mmol/l (159 mg/dl), apolipoprotein B 1.82 g/l, and lipoprotein (a) 0.32 g/l (normal range <0.24 g/l).

Correspondence: Sam Mirzaee (Sam.Mirzaee@monashhealth.org)

Initially submitted January 4, 2019; date of first revision February 17, 2019; accepted for publication March 6, 2019; online publication March 14, 2019.

Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia.

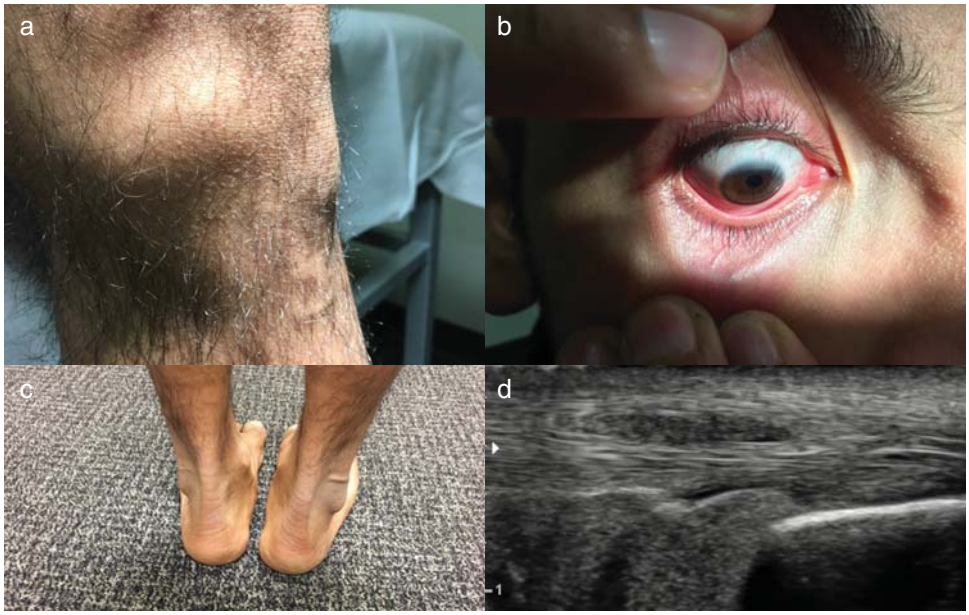


Figure 1. (**a**) Patella tendon xanthoma, (**b**) Arcus cornealis (arrow), (**c**) Achilles tendon thickened on physical examination, and (**d**) Achilles tendon xanthoma on ultrasound (arrow).

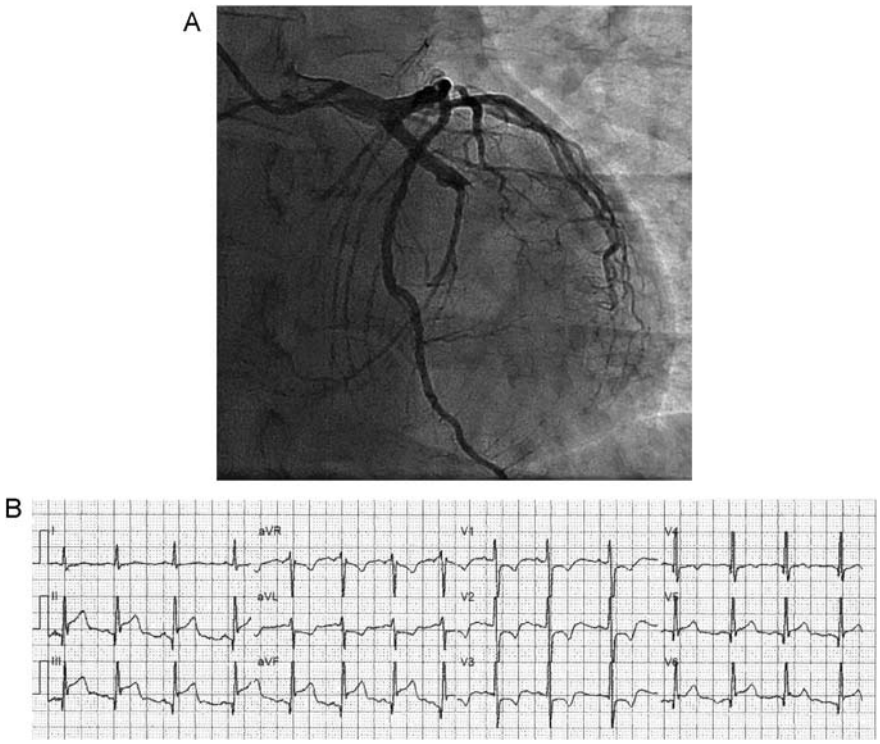


Figure 2. (**a**) Left heart catheterization demonstrating complete occlusion of the left circumflex artery. (**b**) ECG demonstrating inferior and posterior STEMI. ECG, electrocardiogram; STEMI, ST elevation myocardial infarction.

INVESTIGATIONS AND MANAGEMENT

Urgent coronary angiography demonstrated complete occlusion of the proximal left circumflex artery in a left dominant system, with mild-to-moderate coronary artery disease elsewhere (Figure 2). Subsequently, a drug-eluting stent was successfully deployed *via* percutaneous coronary intervention to the culprit lesion. His peri-infarct cardiac biomarkers were substantially elevated with serum creatine kinase peaked at 6,462 units/l (normal <250), and troponin-I levels were more than 82 ng/ml (normal <0.08 ng/ml) indicating an extensive myocardial infarction. This was confirmed by an echocardiogram showing segmental left ventricular dysfunction and resultant left ventricular ejection fraction of 40–45%.

Investigations for secondary hypertension were unremarkable for endocrine etiologies. Renovascular Doppler ultrasound in conjunction with computed tomography angiogram demonstrated significant proximal left renal artery stenosis (RAS) of greater than 60% (Figure 3). The computed tomography angiogram also demonstrated extensive atherosclerotic disease in the "mesenteric arterial system" with an occluded inferior mesenteric artery and 50% stenosis of the proximal superior mesenteric artery; however, he was clinically without symptoms.

Ultrasonography of his Achilles tendon confirmed tendon xanthoma, with the presence of focal thickening and multiple hypoechoic foci within the tendon. By Dutch Lipid Clinical Network Criteria of 21, we diagnosed him with phenotypical FH. He was referred to our lipid clinic for further management of FH as well as family cascade screening. Further, follow-up by nephrology recommended left renal artery angioplasty to treat left RAS.

DISCUSSION

To the best of our knowledge, this is the first case report that illustrates FH as a secondary cause of early-onset hypertension due to left RAS. This case also demonstrates that the absence of an early diagnosis of FH can lead to suboptimal medical management and a sinister but somewhat preventable outcome. In fact, there is evidence that early initiation of statin therapy in patients with FH can reduce their risk to age-matched population levels.[7] The authors wish to highlight that FH is a panvascular disease and its complications are not limited to only early-onset coronary disease but may be extended to early-onset hypertensive renovascular disease and other atherosclerotic diseases such as mesenteric vessel lesions, which should be distinguished from essential hypertension.[8]

The direct association of FH with secondary hypertension has not been well described in the literature. However, limited data report the role that certain renin–angiotensin system genetic polymorphisms can play in accelerating coronary heart disease in patients with FH.[9] Hypertension and hypercholesterolemia are distinct but often coexistent closely related diseases.[10] Hypertension itself accelerates the process of atheroma formation, especially in susceptible individuals.[11] The role of BP in the pathogenesis of atherosclerosis is also evident by the predominance of atheroma formation in high pressure systemic arterial systems over the lower pressure venous and pulmonary arterial systems.[11,12]

Stenosis of the renal arteries, a well-known cause of secondary hypertension, is commonly a result of atherosclerotic plaque, often reported in the proximal to mid portion of the artery.[13] Although a clear benefit of renal artery stenting over optimal medical management has not been observed in non-FH individual with atherosclerotic RAS, there have not been studies focused on an FH population who are potentially more likely to develop early-onset symptomatic RAS.[14–16] Whereas the degree of RAS and hypertension are independent, treatment solely on the basis of degree of RAS may result in further disease progression and eventually renal atrophy.[17,18] The identification of RAS in patients with FH and hypertension may perhaps lend an additional piece of information that could perhaps further optimize the recently developed SAFEHEART risk prediction equation, to determine the long term risk of ASCVD in patients with FH.[19]

In summary, we believe that our case demonstrates several clinical points to inform optimal management of patients with FH: (i) Early-onset hypertension (<30 years of age) should prompt clinicians to consider further investigation of renovascular pathologies, (ii) Patients with premature atherosclerotic RAS (male <55 and female <60 years of age) should be screened for FH, (iii) FH is a systemic panvascular disease and a high index of clinical suspicion for subclinical atherosclerosis is essential in the management of these patients, and (iv) Early intense pharmacological in addition

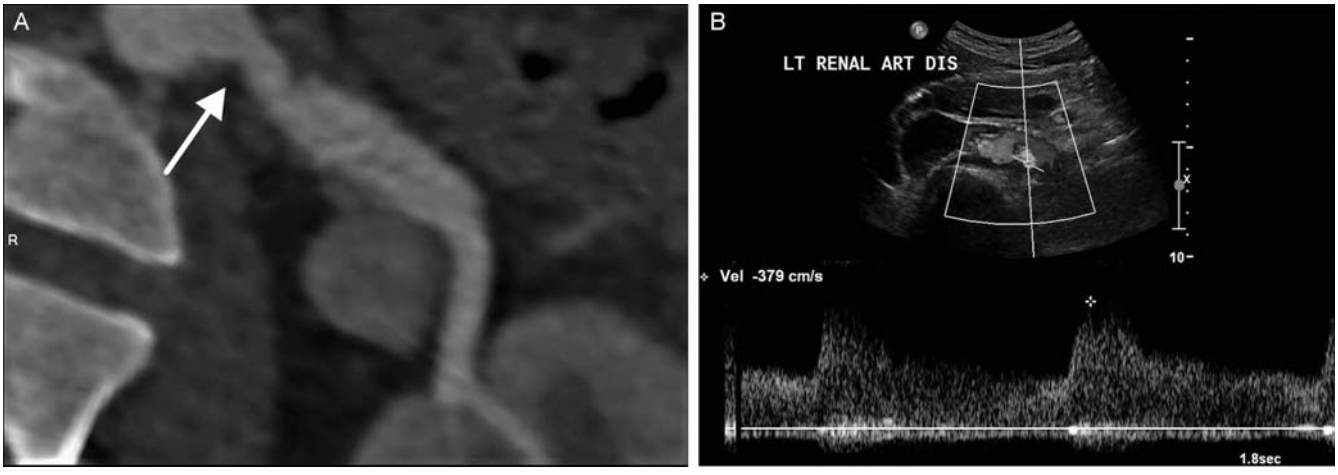


Figure 3. (**a**) CT angiogram demonstrating severe proximal left renal artery stenosis (arrow). (**b**) Doppler waveforms study of the same artery showing high velocities (3.8 m/s) indicating severe RAS. CT angiogram, computed tomography angiogram; RAS, renal artery stenosis.

to diet and lifestyle modification is the key lipid-lowering approach in patients with FH to prevent further development of serious atherosclerotic complications.

DISCLOSURE

The authors of this article have no conflicts of interest to declare.

REFERENCES

1. Yamamoto A, Kamiya T, Yamamura T, Yokoyama S, Horiguchi Y, Funahashi T, Kawaguchi A, Miyake Y, Beppu S, Ishikawa K. Clinical features of familial hypercholesterolemia. *Arteriosclerosis* 1989; 9:I66–I74.
2. Hopkins PN, Toth PP, Ballantyne CM, Rader DJ; National Lipid Association Expert Panel on Familial Hypercholesterolemia. Familial hypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from the national lipid association expert panel on familial hypercholesterolemia. *J Clin Lipidol* 2011; 5:S9–S17.
3. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. *J Clin Endocrinol Metab* 2012; 97:3956–3964.
4. Defesche JC, Lansberg PJ, Umans-Eckenhausen MA, Kastelein JJ. Advanced method for the identification of patients with inherited hypercholesterolemia. *Semin Vasc Med* 2004; 4:59–65.
5. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. *Heart* 2016; 102:1003–1008.
6. Taylor A, Wang D, Patel K, Whittall R, Wood G, Farrer M, Neely RD, Fairgrieve S, Nair D, Barbir M, Jones JL, Egan S, Everdale R, Lolin Y, Hughes E, Cooper JA, Hadfield SG, Norbury G, Humphries SE. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. *Clin Genet* 2010; 77:572–580.
7. Versmissen J, Oosterveer DM, Yazdanpanah M, Defesche JC, Basart DC, Liem AH, Heeringa J, Witteman JC, Lansberg PJ, Kastelein JJ, Sijbrands EJ. Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *BMJ* 2008; 337:a2423.
8. Simon N, Franklin SS, Bleifer KH, Maxwell MH. Clinical characteristics of renovascular hypertension. *JAMA* 1972; 220:1209–1218.
9. Wierzbicki AS, Lambert-Hammill M, Lumb PJ, Crook MA. Renin–angiotensin system polymorphisms and coronary events in familial hypercholesterolemia. *Hypertension* 2000; 36:808–812.
10. Senarathne R, Dissanayake N, Hettiaratchi U, Athiththan L. Assessment of lipid profile in hypertensive and non-hypertensive males: a case control study. *J Clin Lipidol* 2016; 10:704.
11. Alexander RW. Theodore Cooper memorial lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension* 1995; 25:155–161.
12. Kannel WB. Hypertension and other risk factors in coronary heart disease. *Am Heart J* 1987; 114:918–925.
13. Dworkin LD, Cooper CJ. Clinical practice. Renal-artery stenosis. *N Engl J Med* 2009; 361:1972–1978.
14. Trinquart L, Mounier-Vehier C, Sapoval M, Gagnon N, Plouin PF. Efficacy of revascularization for renal artery stenosis caused by fibromuscular dysplasia: a systematic review and meta-analysis. *Hypertension* 2010; 56:525–532.
15. Safian RD, Textor SC. Renal-artery stenosis. *N Engl J Med* 2001; 344:431–442.
16. Balk E, Raman G, Chung M, Ip S, Tatsioni A, Alonso A, Chew P, Gilbert SJ, Lau J. Effectiveness of management strategies for renal artery stenosis: a systematic review. *Ann Intern Med* 2006; 145:901–912.
17. Caps MT, Perissinotto C, Zierler RE, Polissar NL, Bergelin RO, Tullis MJ, Cantwell-Gab K, Davidson RC, Strandness DE Jr. Prospective study of atherosclerotic disease progression in the renal artery. *Circulation* 1998; 98:2866–2872.
18. Caps MT, Zierler RE, Polissar NL, Bergelin RO, Beach KW, Cantwell-Gab K, Casadei A, Davidson RC, Strandness DE Jr. Risk of atrophy in kidneys with atherosclerotic renal artery stenosis. *Kidney Int* 1998; 53:735–742.
19. Perez de Isla L, Alonso R, Mata N, Fernandez-Perez C, Muniz O, Diaz-Diaz JL, Saltijeral A, Fuentes-Jimenez F, de Andres R, Zambon D, Piedecausa M, Cepeda JM, Mauri M, Galiana J, Brea A, Sanchez Munoz-Torrero JF, Padro T, Argueso R, Miramontes-Gonzalez JP, Badimon L, Santos RD, Watts GF, Mata P. Predicting cardiovascular events in familial hypercholesterolemia: the SAFEHEART registry (Spanish familial hypercholesterolemia cohort study). *Circulation* 2017; 135:2133–2144.

Journal of Clinical Lipidology (2018) ■, ■

Journal of Clinical Lipidology

Letter to the Editor

Familial hypercholesterolemia and cardiovascular risk stratification

Al-Rasadi et al.[1] recently published a multicentre report of prevalence and cardiovascular outcome of familial hypercholesterolemia (FH) among an Arab population with acute coronary syndrome (ACS) in the Persian Gulf region. It is clear the prevalence and the risk of early onset cardiovascular disease are considerably higher in FH patients and these data are in line with the existing literature.[2] In terms of risk of recurrent cardiovascular events, FH patients have higher rate of recurrence with an approximately two fold increase within the first year of discharge implying a poorer prognosis.[3] A recent follow-up study in Chinese patients with FH having premature ST-segment elevation myocardial infarction revealed a higher occurrence of multivessel disease and impaired ventricular function in FH, which was translated to a statistically significant difference in Killip class as a predictor of mortality.[4]

These findings are particularly interesting because in Al-Rasadi et al.'s study outcome, there was a discrepancy in ACS risk assessment via The Global Registry for Acute Coronary Events score in FH group versus non-FH with erroneously lower risk of further cardiac events in FH (P value $<$ .001), while after multivariate adjustment, FH became associated with worse prognosis in composite cardiovascular outcomes.[1]

Sharifi et al.[5] has suggested traditional risk stratification tools are insufficient to correctly guide clinicians to optimize primary prevention of cardiovascular disease in patients with FH. Similarly, the Global Registry for Acute Coronary Events score has been developed to stratify the risk of recurrent events and mortality in ACS patients without FH.[6] We wish to highlight that current cardiovascular risk stratification tools have been established for non-FH patients; thus, they may not only be inadequate for FH individuals, they may in fact mislead clinicians. Cardiovascular disease is the major cause of morbidity and mortality, and associated with important consequences in the FH population, there is an imperative for a dedicated novel cardiovascular risk stratification method to be developed for both primary and secondary prevention in FH patients.

Sam Mirzaee, MD, MPH
James D. Cameron, MBBS, MD, BE (Elec), MEngSc
Monash Cardiovascular Research Centre
MonashHeart, Monash Health
Monash University, Melbourne, Australia

https://doi.org/10.1016/j.jacl.2018.07.002

References

1. Al-Rasadi K, Al-Zakwani I, Alsheikh-Ali AA, et al. Prevalence, management, and outcomes of familial hypercholesterolemia in patients with acute coronary syndromes in the Arabian Gulf. *J Clin Lipidol*. 2018;12(3):685–692.e2.
2. Pang J, Poulter EB, Bell DA, et al. Frequency of familial hypercholesterolemia in patients with early-onset coronary artery disease admitted to a coronary care unit. *J Clin Lipidol*. 2015;9(5):703–708.
3. Nanchen D, Gencer B, Muller O, et al. Prognosis of patients with familial hypercholesterolemia after acute coronary syndromes. *Circulation*. 2016;134(10):698–709.
4. Auckle R, Su B, Li H, et al. Familial hypercholesterolemia in Chinese patients with premature ST-segment-elevation myocardial infarction: Prevalence, lipid management and 1-year follow-up. *PLoS One*. 2017;12(10):e0186815.
5. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. *Heart*. 2016;102(13):1003–1008.
6. Roffi M, Patrono C, Collet J-P, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European society of cardiology (ESC). *Eur Heart J*. 2016;37(3):267–315.

References

1. Al-Rasadi K, Al-Zakwani I, Alsheikh-Ali AA, et al. Prevalence, management, and outcomes of familial hypercholesterolemia in patients with acute coronary syndromes in the Arabian Gulf. *J Clin Lipidol*. 2018;12(3):685–692.e2.
2. Pang J, Poulter EB, Bell DA, et al. Frequency of familial hypercholesterolemia in patients with early-onset coronary artery disease admitted to a coronary care unit. *J Clin Lipidol*. 2015;9(5):703–708.
3. Nanchen D, Gencer B, Muller O, et al. Prognosis of patients with familial hypercholesterolemia after acute coronary syndromes. *Circulation*. 2016;134(10):698–709.
4. Auckle R, Su B, Li H, et al. Familial hypercholesterolemia in Chinese patients with premature ST-segment-elevation myocardial infarction: Prevalence, lipid management and 1-year follow-up. *PLoS One*. 2017;12(10):e0186815.
5. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. *Heart*. 2016;102(13):1003–1008.
6. Roffi M, Patrono C, Collet J-P, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European society of cardiology (ESC). *Eur Heart J*. 2016;37(3):267–315.

Use of cardiovascular risk scores in acute coronary syndrome patients with familial hypercholesterolemia

We read with interest the "Letter to the Editor" written by Mirzaee and Cameron[1] who commented about the discrepancy of the Global Registry for Acute Coronary Events (GRACE) score in its cardiovascular risk stratification in the Arabian Gulf population with acute coronary syndrome with familial hypercholesterolemia (FH).[2] It's important to note that the GRACE risk score is a validated clinical risk score to predict mortality in acute coronary syndrome populations and not necessarily in FH subjects.[3,4] Furthermore, the atherosclerotic vascular disease (ASCVD) outcome in the study by Al-Rasadi *et al*[2] was a composite mixture of not only mortality but also myocardial infarction, transient ischemic attack, and stroke. In addition, the asymptomatic nature of subclinical FH coupled with young age might have also contributed to the lower GRACE risk scores and hence its discrepancy in its correlation with ASCVD outcomes in such populations. Hence, we agree with the authors,[1] and in line with published literature,[5] that the current traditional stratification risk scores, including GRACE risk score, have lower discriminative ability to correctly predict cardiovascular outcomes in patients with FH.

de Isla et al, from the SAFEHEART Registry, have proposed an improved risk stratification criterion that predicted cardiovascular events in FH patients.[6] Other risk stratification tools such as genetic (*LDLR*, APOB, and PCSK9 mutations),[7] biochemical (lipoprotein(a)),[8] and imaging techniques (including carotid intima–media thickness[9] and calcium score[10]) have been suggested to produce more reliable information in cardiovascular risk prediction in patients with FH. However, more research is warranted to determine their usefulness in such patients.

Khalid Al-Rasadi, MD
Department of Biochemistry
Sultan Qaboos University Hospital
Muscat, Oman
Ibrahim Al-Zakwani, PhD
Department of Pharmacology & Clinical Pharmacy
College of Medicine & Health Sciences
Sultan Qaboos University
Muscat, Oman

https://doi.org/10.1016/j.jacl.2018.07.001

References

1. Mirzaee S, Cameron JD. Familial hypercholesterolemia and cardiovascular risk stratification. *J Clin Lipidol*. 2018.
2. Al-Rasadi K, Al-Zakwani I, Alsheikh-Ali AA, et al. Prevalence, management, and outcomes of familial hypercholesterolemia in patients with acute coronary syndromes in the Arabian Gulf. *J Clin Lipidol*. 2018;12:685–692.
3. Fox KA, Dabbous OH, Goldberg RJ, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observation study (GRACE). *BMJ*. 2006;333:1091.
4. Yusufali A, Zubaid M, Al-Zakwani I, et al. Validation of the GRACE risk score for hospital mortality in patients with acute coronary syndrome in the arab middle east. *Angiology*. 2011;62:390–396.
5. Sharifi M, Rakhit RD, Humphries SE, Nair D. Cardiovascular risk stratification in familial hypercholesterolaemia. *Heart*. 2016;102:1003100–1003108.
6. De Isla LP, Alonso R, Mata N, et al. Predicting cardiovascular events in familial hypercholesterolemia: the safeheart registry (spanish familial hypercholesterolemia cohort study). *Circulation*. 2017;135:2133–2144.
7. Humphries SE, Whittall RA, Hubbart CS, et al. Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. *J Med Genet*. 2006;43:943–949.
8. Nenseter MS, Lindvig HW, Ueland T, et al. Lipoprotein(a) levels in coronary heart disease-susceptible and -resistant patients with familial hypercholesterolemia. *Atherosclerosis*. 2011;216:426–432.
9. Junyent M, Gilabert R, Jarauta E, et al. Impact of low-density lipoprotein receptor mutational class on carotid atherosclerosis in patients with familial hypercholesterolemia. *Atherosclerosis*. 2010;208:437–441.
10. ten Kate GL, ten Kate GJ, van den Oord SC, et al. Carotid plaque burden as a measure of subclinical coronary artery disease in patients with heterozygous familial hypercholesterolemia. *Am J Cardiol*. 2013;111:1305–1310.

Journal of Clinical Lipidology (2018) 12, 1549–1551

Journal of Clinical Lipidology

Letters to the Editor

Coronary computed tomography angiogram in familial hypercholesterolemia: A double edge sword

Sam Mirzaee, MD, MPH*
James D. Cameron, MBBS, MD, BE (Elec), MEngSc
Monash Cardiovascular Research Centre
MonashHEART, Monash Health
Monash University
Melbourne, Australia
*Corresponding author. Monash Health Cardiology, 246 Clayton Road, Clayton, VIC, Melbourne, Australia.
E-mail address: sam.mirzaee@monashhealth.org (S. Mirzaee)

https://doi.org/10.1016/j.jacl.2018.09.014

In the April 2018 issue of the journal, Perez de Isla et al.[1] published a multicentre, long-term prospective cohort report of coronary computed tomography angiogram (CTA) outcome in an asymptomatic molecularly defined population of heterozygous familial hypercholesterolemia (FH) in Spain. FH is a hereditary lipid disorder often identified at a young age resulting in substantial increase in risk of premature coronary atherosclerotic disease. However, there is heterogeneity among the FH population from completely normal to severe coronary disease, which might be related to several factors including the presence of conventional risk factors, namely smoking and hypertension, elevated lipoprotein (a), and increased body mass index.[2]

It is generally agreed that coronary CTA is a safe, noninvasive modality to detect subclinical coronary atherosclerosis in FH population. Perez de Isla et al. described that patients with more advanced coronary disease received more lipid-lowering therapies (LLTs) after CTA result, which is in line with the large body of current evidence.[1,3] Interestingly, they showed smoking cessation was greater in FH patients if CTA showed no significant coronary artery disease. Use of optimal lipid-lowering therapy ($P = .01$), and change in low-density lipoprotein cholesterol levels ($P = .005$) in FH with no significant coronary artery disease on CTA, however was attenuated. This can be explained by two reasons: first, less reinforcement for intensified LLTs by clinicians and second, lower adherence to therapy and lifestyle modifications by patients after a coronary CTA not showing significant disease. This report is specially important because FH is a panvascular disease, and its ramification is not limited to lipoprotein metabolism and early onset coronary disease but may be extended to hemostatic balance[4] increasing the risk of stroke, transient ischemic attack, and peripheral vascular disease; therefore, intensive LLTs should not be compromised by normal coronary CTA report.[5]

Irrespective of the presence or absence of atherosclerosis in coronary CTA, patients with FH should remain on intensive preventive interventions including receiving both combined pharmacotherapy and therapeutic lifestyle modification.

References

1. Perez de Isla L, Alonso R, Muniz-Grijalvo O, et al. Coronary computed tomographic angiography findings and their therapeutic implications in asymptomatic patients with familial hypercholesterolemia. Lessons from the SAFEHEART study. *J Clin Lipidol*. 2018;12:948–957.
2. Pérez de Isla L, Alonso R, Mata N, et al. Predicting cardiovascular events in familial hypercholesterolemia: The SAFEHEART Registry. *Circulation*. 2017;135:2133–2144.
3. Shapiro MD, Fazio S. "Taking a look under the hood"-imaging the phenotypic heterogeneity of familial hypercholesterolemia. *J Clin Lipidol*. 2018;12:1095–1098.
4. Martinelli N, Girelli D, Lunghi B, et al. Polymorphisms at LDLR locus may be associated with coronary artery disease through modulation of coagulation factor VIII activity and independently from lipid profile. *Blood*. 2010;116(25):5688–5697.
5. Toell T, Mayer L, Pechlaner R, et al. Familial hypercholesterolaemia in patients with ischaemic stroke or transient ischaemic attack. *Eur J Neurol*. 2018;25(2):260–267.

Response to letter entitled "Coronary computed tomography angiogram in familial hypercholesterolemia: A double edge sword" by Dr. Sam Mirzaee and Dr. James D Cameron

We have read with deep attention the letter from doctors Mirzaee and Cameron.[1] We fully agree with the phenotypic variability and, subsequently, with the risk heterogeneity present in patients with familial hypercholesterolemia (FH), as well as with the role that other risk factors may have, as we can see in our recently published article that

the authors refer to.[2] And, of course, we also totally agree with the fact that, regardless of the presence or absence of atherosclerosis in coronary computed tomography angiogram, patients with FH should be pharmacologically and nonpharmacologically intensively treated.

The authors define two edges: one is good but the other is bad. However, we would like to turn these two edges, posed by the authors, into two good edges. They are good, from our point of view, because they are useful for patients with FH. One of the edges would be the ability to reinforce the idea of illness and the need for intense treatment in those patients in whom atherosclerosis is found in coronary computed tomography, as mentioned by doctors Mirzaee and Cameron. But the other one would be also a good one, because we cannot disregard its utility when coronary disease is not found. It is well established that the absence of atherosclerotic disease found in coronary computed tomography angiogram is associated with a better cardiovascular prognosis.[3] So, this diagnostic technique could be of great help when selecting which patients should benefit from more intensive and at the same time more expensive lipid-lowering treatments.[4]

Leopoldo Pérez de Isla, MD, PhD*
Cardiology Department
Hospital Clínico San Carlos
IDISSC, Universidad Complutense
Madrid, Spain
Fundación Hipercolesterolemia Familiar
Madrid, Spain
*Corresponding author. Hospital Clínico San Carlos. Unidad de Imagen Cardiovascular, C/ Profesor Martín Lagos s/n., 28040, Madrid, Spain.
Rodrigo Alonso, MD, PhD
Fundación Hipercolesterolemia Familiar
Madrid, Spain
Nutrition Department, Clínica las Condes
Santiago de Chile, Chile
Pedro Mata, MD, PhD
Fundación Hipercolesterolemia Familiar
Madrid, Spain
E-mail address: leopisla@hotmail.com

https://doi.org/10.1016/j.jacl.2018.10.001

References

1. Mirzaee S, Cameron JD. Letter: coronary computed tomography angiogram in familial hypercholesterolemia; a double edge sword. *J Clin Lipidol*. 2018;12:1328–1329.
2. Perez de Isla L, Alonso R, Mata N, et al. Predicting cardiovascular events in familial hypercholesterolemia: The SAFEHEART Registry (Spanish Familial Hypercholesterolemia Cohort Study). *Circulation*. 2017;135:2133–2144.
3. Bittencourt MS, Hulten E, Ghoshhajra B, et al. Prognostic value of nonobstructive and obstructive coronary artery disease detected by coronary computed tomography angiography to identify cardiovascular events. *Circ Cardiovasc Imaging*. 2014;7:282–291.
4. Santos RD, Gidding SS, Hegele RA, et al, International Atherosclerosis Society (IAS) Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolemia: implications for clinical management. *Lancet Diabetes Endocrinol*. 2016;4:850–861.

Extremely high levels of high-density lipoprotein cholesterol and increased risk of cardiovascular mortality

I have read with interest a report by Hirata et al., who evaluated the risk of extremely high levels of serum high-density lipoprotein cholesterol (HDL-C) for cardiovascular events.[1] The adjusted hazard ratios (95% confidence intervals) of subjects with serum HDL-C≧2.33 mmol/L for cardiovascular mortality were 2.60 (1.1–5.16) in men and 2.02 (0.81–5.01) in women, respectively. Ischemic stroke mortality and stroke mortality in men were also significantly associated with extremely high levels of serum HDL-C. I have two concerns with this study.

First, the authors recognized that the risk of extremely high levels of serum HDL-C for cardiovascular mortality was evident in current drinkers. The cholesteryl ester transfer protein is stimulated by light-to-moderate alcohol consumption and is suppressed by heavy alcohol consumption, and they speculated that alcohol consumption might affect the relationship between extremely high levels of serum HDL-C and cardiovascular mortality. I suppose that a sex difference on the association would partly be related to the difference in the frequency and the amount of alcohol consumption by sex. Sex difference in several risk factors for cardiovascular mortality would relate to the magnitude of serum HDL-C contribution for cardiovascular mortality.

Second, discussion on the components of serum HDL-C for the risk of cardiovascular mortality would be useful. HDL functionality with different subclass has been traditionally discussed, and HDL-C subclass measurement might be considered in addition to the simple measurement of HDL-C amount.[2] HDL-C can be separated into two distinct subclasses, HDL-C2 (large) and HDL-C3 (small), by agarose gel electrophoresis.[3] There is a structural association between HDL-C3 and sphingosine-1-phosphate, which is a cardioprotective lipid,[4] and myocardial infarction patients with low levels of HDL-C3 have an increased risk of long-term clinical events.[5] Taken together, a risk assessment of extremely high levels of serum HDL-C for cardiovascular events should be conducted by considering both quality and quantity of serum HDL-C.

Financial disclosure

The authors have no conflicts of interest to disclose.

Tomoyuki Kawada, MD
Department of Hygiene and Public Health
Nippon Medical School
Tokyo, Japan

https://doi.org/10.1016/j.jacl.2018.10.010

The Role of Nutrition and Environmental Factors in Management of Familial Hypercholesterolemia

Author:
Sam Mirzaee MD, MPH, FRACP[1]; James D. Cameron MBBS, MD, BE (Elec) MEngSc1; Stephen J. Nicholls MBBS, PhD, FRACP [1]

[1] *Monash Cardiovascular Research Centre, MonashHEART, Monash Health, Monash University, Melbourne, Australia,*

INTRODUCTION

Heterozygous Familial Hypercholesterolemia (HeFH) is a common autosomal dominant hereditary disorder of lipoprotein metabolism characterised by high plasma concentrations of low-density lipoprotein cholesterol (LDL-C), and high risk of premature atherosclerotic cardiovascular disease (ASCVD).[1] Recent evidence suggests that the prevalence of HeFH is as high as 1 in 200-250 in the general population, and is often both underdiagnosed and undertreated by health care providers.[2, 3] There is heterogeneity in phenotypical expression and development of ASCVD among FH patients with substantial individual variation. It appears that significant elevation of plasma LDL-C is not always be seen in every HeFH patient nor is LDL-C level closely correlated with manifestation of ASCVD.

Computed tomography coronary angiography studies have demonstrated that atheroma plaques start to develop in males and females with HeFH in their early twenties and thirties, respectively.[4] However, several environmental factors in conjunction with other cardiovascular risk factors contribute to the progression of plaque formation and prediction of ASCVD events in HeFH. Several genetic studies in familial hypercholesterolemia (FH) have established a wide range of LDL-C levels with distinct cardiovascular outcomes (Table 1A-B).[5, 6] This phenotypic discrepancy could be influenced not only by different genetic composition but also by gender, body mass index (BMI), habits, hypertension, smoking, insulin resistance and possibly other coexisting forms of lipid disorders.[7]

A-Phenotypical Familial Hypercholesterolemia	
Consistent	Significantly elevated LDL-C levels with severe ASCVD
Inconsistent	Significantly elevated LDL-C levels (3-4 times above the normal) without ASCVD OR Normal to slightly elevated LDL-C levels with substantial ASCVD OR Significantly elevated Lipoprotein (a) [3-4 times] with normal to slightly elevated LDL-C levels with substantial ASCVD
B-Genotypes of Familial Hypercholesterolemia	
Homozygous	Homozygous for a mutation in one of the known genes known to cause FH 3
Heterozygous	Heterozygous for a mutation in one of the known genes to cause FH
Mixed	Heterozygous for two different mutations in the same or different known genes to cause FH
Unknown	No causative mutation could be detected after screening all candidate genes known to cause FH

TABLE 1
Variability in manifestation of familial hypercholesterolemia [3-5, 11]

FH is linked to several known gene mutations including the low density lipoprotein receptor (LDLR) as the most common defect followed by abnormalities in apolipoprotein-B 100 (Apo-B), proprotein convertase subtilisin/kexin type 9 (PCSK9), and Low-density lipoprotein receptor adapter protein (LDLR-AP) [Figure 1]. [8-10] Other genetic factors that have been described as a being risk factor for the development of ASCVD in FH include Apolipoprotein-E2 (APO- E2) genotype and lipoprotein (a) [Lp(a)].[11] The levels of Lp(a) are independent risk factors for the development of ASCVD in FH. Negative genetic testing in FH does not exclude the diagnosis. This could be due to a polygenetic nature of the disease including both known and unknown genes, high Lp(a) or phenocopies including sitosteorolemia and lysosomal acid lipase deficiency.[12] This work highlights the significance of a holistic approach in management of FH by considering environmental factors and nutrition as management options to alter the development of ASCVD and occurrence of future events.

THE SIGNIFICANCE OF GENETIC TESTING IN FAMILIAL HYPERCHOLESTEROLEMIA

Although genetic testing may provide a definite diagnosis of FH if a pathological mutation is detected, negative genetic testing does not exclude FH. The diagnosis of FH can be made using phenotypic criteria that include family history, clinical history, and physical examination looking for tendinous xanthoma, arcus cornealis before 45 years of age and LDL-C levels in addition to genetic testing.[13] Genetic testing is particularly important in the diagnosis of equivocal cases, children with elevated LDL-C, providing prognostic value, improving adherence to treatment and implications for therapeutic choices and assisting cascade screening.[12]

FH POPULATIONS AND DIATARY ADVICE

Since every individual is genetically unique, the biochemical process is also variable person by person. Similar to most genetic diseases whose pathogenesis are influenced by nutritional factors diet can impact on the course of FH. Dietary modification, reducing the intake of cholesterol rich foods and saturated fats, should be the first line recommendation in the treatment of HeFH. International guidelines recommend FH patient to reduce total fat to 25-35% of energy intake, saturated fat < 7% of total fat, plant stanol or sterol esters 2 g/day, and soluble fibre 10-20 g/day.[14]

Adoptation of a healthy diet will have a lifelong benefit in heart disease prevention for all FH patients. However, it appears for some FH patients including young children, pregnant women, and patients who have the genetic mutation but having a lipid profile below the pharmacotherapy threshold it would be more beneficial.[14]

THE IMPACT OF LIFESTYLE ON THE COURSE OF FAMILIAL HYPERCHOLESTEROLEMIA

The phenotypical expression of heterozygous FH is not only variable in patients with different gene mutation but can be highly inconsistent among FH cases carrying the same gene mutation. This variability might be due to multifactorial aetiologies including anthropometrics, age, the presence of other cardiovascular risks, high fat or high calorie diet, smoking, other components of lipid profile such as HDL-cholesterol and triglyceride concentration, physical activities in addition to modifying genes affecting lipoprotein metabolism.[15] Therefore, adequate physical activity and energy intake to achieve and maintain healthy body weight; limitation of alcohol consumption; recommendation to avoid the use of any tobacco products play a pivotal role in the management of FH.

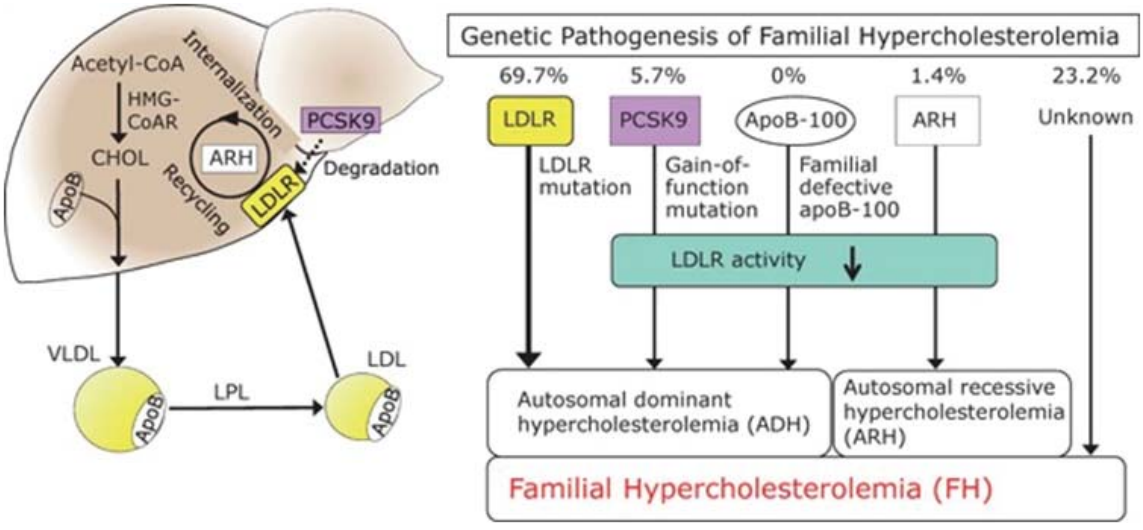


Figure 1 - Genetic pathogeneses in FH[10]

FUTURE RESEARCH IN THE NUTRITIONAL MANAGEMENT OF FAMILIAL HYPERCHOLESTEROLEMIA

In the era of novel lipid lowering therapies and moving towards "personalised medicine", evaluation of clinical heterogeneity and details of genetic mutations among individuals with FH requires much greater understanding.[16] There is an unmet need for studying the role of nutrigenomics in this high risk population, to establish a "personalised diet", and subsequently optimising both prevention and treatment.

CONCLUSION

Familial Hypercholesterolemia is a common underdiagnosed hereditary lipid disorder causing early onset ASCVD. Variable clinical manifestations of FH are likely due to the multifactorial causes including genetic and non-genetic elements which impact on the course of this disease. Furthermore, it is imperative for clinicians to acknowledge modifiable environmental factors impact on FH and to adopt an holistic approach in the management of this treatable disease.

References

1. *Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease : Consensus Statement of the European Atherosclerosis Society. European Heart Journal. 2013;34(45):3478-90.*

2. *Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. International journal of cardiology. 2014;171(3):309-25.*

3. *Faggiano P, Pirillo A, Griffo R, Ambrosetti M, Pedretti R, Scorcu G, et al. Prevalence and management of familial hypercholesterolemia in patients with coronary artery disease: The heredity survey. International journal of cardiology. 2018;252:193-8.*

4. *Miname MH, Ribeiro MS, 2nd, Parga Filho J, Avila LF, Bortolotto LA, Martinez LR, et al. Evaluation of subclinical atherosclerosis by computed tomography coronary angiography and its association with risk factors in familial hypercholesterolemia. Atherosclerosis. 2010;213(2):486-91.*

5. *Raal FJ, Sjouke B, Hovingh GK, Isaac BF. Phenotype diversity among patients with homozygous familial hypercholesterolemia: A cohort study. Atherosclerosis. 2016;248:238-44.*

6. *Sjouke B, Kusters DM, Besseling J, Hovingh GK, Kastelein JJP, Kindt I, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype–phenotype relationship, and clinical outcome. European Heart Journal. 2014;36(9):560-5.*

7. *Jansen ACM, van Aalst-Cohen ES, Tanck MW, Trip MD, Lansberg PJ, Liem AH, et al. The contribution of classical risk factors to cardiovascular disease in familial hypercholesterolaemia: data in 2400 patients. Journal of Internal Medicine. 2004;256(6):482-90.*

8. *Garcia CK, Wilund K, Arca M, Zuliani G, Fellin R, Maioli M, et al. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science. 2001;292(5520):1394-8.*

9. *Brown MS, Goldstein JL. Expression of the familial hypercholesterolemia gene in heterozygotes: mechanism for a dominant disorder in man. Science. 1974;185(4145):61-3.*

10. *Mabuchi, Hiroshi. "Half a Century Tales of Familial Hypercholesterolemia (FH) in Japan." Journal of atherosclerosis and thrombosis vol. 24,3 (2017): 189-207.*

11. *Duly EB, Ward AJ, Kirk CW, Nevin NC, Trinick TR, Nicholls DP, et al. APO E Genotype and Familial Hypercholesterolaemia. Annals of Clinical Biochemistry. 1997;34(5):534-6.*

12. *12.Sturm AC, Knowles JW, Gidding SS, Ahmad ZS, Ahmed CD, Ballantyne CM, et al. Clinical Genetic Testing for Familial Hypercholesterolemia. JACC, Scientific Expert Panel. 2018;72(6):662-80.*

13. *Watts GF, Sullivan DR, Poplawski N, van Bockxmeer F, Hamilton-Craig I, Clifton PM, et al. Familial hypercholesterolaemia: a model of care for Australasia. Atherosclerosis Supplements. 2011;12(2):221-63.*

14. *Goldberg AC, Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, Robinson JG, et al. Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. Journal of clinical lipidology. 2011;5(3):133-40.*

15. *Koeijvoets KC, van der Net JB, Dallinga-Thie GM, Steyerberg EW, Mensink RP, Kastelein JJ, et al. ABCG8 gene polymorphisms, plasma cholesterol concentrations, and risk of cardiovascular disease in familial hypercholesterolemia. Atherosclerosis. 2009;204(2):453-8.*

16. *Soutar AK, Naoumova RP. Mechanisms of Disease: genetic causes of familial hypercholesterolemia. Nature Clinical Practice Cardiovascular Medicine. 2007;4:214.*

American Journal of Cardiovascular Drugs
https://doi.org/10.1007/s40256-019-00363-3

CURRENT OPINION

High-Dose Omega-3 Fatty Acids in Cardiovascular Prevention: Finally Living Up to Their Potential?

Adam J. Nelson[1] · **Sam Mirzaee**[2] · **Stephen J. Nicholls**[2]

Abstract
Despite the widespread use of statins in the setting of high cardiovascular risk, many patients continue to experience clinical events. This highlights the need to identify additional therapeutic strategies for high-risk patients. Interest in the use of omega-3 polyunsaturated fatty acids to prevent cardiovascular disease has been high for several decades. Despite promising results from before the statin era, many clinical trials have produced disappointing findings regarding products containing conventional doses of omega-3 fatty acids. More recent clinical trials using high doses of omega-3 fatty acids in targeted populations have suggested potential benefit when targeting the risk driven by atherogenic dyslipidemia. We review the clinical implications of completed and ongoing trials.

Key Points

High dose EPA reduced cardiovascular events in the REDUCE-IT trial.

This provides an opportunity to target high triglycerides in cardiovascular prevention.

The ongoing STRENGTH trial (NCT02104817) is testing the benefit of triglyceride lowering with omega-3 carboxylic acid (eicosapentaenoic acid 75%, docosahexaenoic acid 25%).

1 Introduction

Over the last three decades, randomized controlled trials have consistently demonstrated that lowering levels of low-density lipoprotein cholesterol (LDL-C) reduces cardiovascular event rates in primary and secondary prevention [1]. Early findings with statin agents have been extended to the use of high-intensity statins, ezetimibe [2], and proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors [3, 4], all demonstrating a direct relationship between incremental LDL-C lowering and cardiovascular benefit. As a result, treatment guidelines have increasingly emphasized the importance of intensive lipid lowering in approaches to cardiovascular prevention. However, despite widespread use of these agents, many cardiovascular events continue to occur [5]. This suggests an ongoing need to develop additional therapeutic strategies to further reduce cardiovascular risk.

2 Atherogenic Dyslipidemia and Cardiovascular Risk

Increasing evidence implicates a range of additional lipid and lipoprotein parameters, beyond LDL-C, in atherosclerosis and cardiovascular risk. Population studies demonstrate that both fasting and non-fasting triglyceride levels are associated with prospective cardiovascular risk [6]. This is supported by observations from Mendelian randomization that factors involved in the metabolism of triglyceride-rich lipoproteins are associated with cardiovascular risk [7, 8], implicating these particles in the causal pathway for atherosclerosis. Cellular studies suggest that triglyceride-rich lipoproteins promote inflammatory, oxidative, and thrombotic pathways in atherosclerosis [9–11], whereas transgenic

✉ Stephen J. Nicholls
stephen.nicholls@monash.edu

[1] Duke Clinical Research Institute, Durham, NC, USA

[2] Monash Cardiovascular Research Centre, Monash University, 246 Clayton Road, Clayton, VIC 3168, Australia

Published online: 04 September 2019

mouse models aimed at increasing plasma triglycerides have an adverse effect on plaque burden [12].

Hypertriglyceridemia has become an increasingly prevalent risk factor by virtue of the rise in abdominal obesity and insulin resistance. This is evidenced by an increase in circulating triglyceride-rich lipoproteins, not only in the fasting state but also during the postprandial period. Following meals, chylomicron and remnant particles increase, with the latter thought to be particularly atherogenic. This is supported by reports that genetic polymorphisms associated with higher remnant cholesterol levels are also associated with cardiovascular risk [13] and that remnant cholesterol levels predict ongoing plaque progression in statin-treated patients [14]. It is therefore likely that any impact of triglyceride-rich lipoproteins in the atherosclerotic disease process is likely to become more problematic as a driver of cardiovascular risk.

In parallel, interest in the ability of high-density lipoproteins (HDLs) to protect against atherosclerosis has been considerable. Population studies demonstrate an inverse relationship between HDL cholesterol (HDL-C) and cardiovascular risk [15], although this risk is curvilinear, largely driven by the increased risk at low HDL-C levels [16]. Preclinical studies demonstrate that HDLs possess functional properties influencing cholesterol transport [17], inflammation [18], oxidation [19], and thrombosis [20] that may underscore the favorable effects of HDL-targeted interventions in animal models of atherosclerosis [21]. This is further supported by contemporary observations that quantitative and qualitative measures of HDL are associated with residual cardiovascular risk, even in the setting of intensive lipid lowering [22, 23]. Given that low HDL-C levels are commonly associated with hypertriglyceridemia, particularly in the setting of obesity and insulin resistance [24], this combined phenotype of atherogenic dyslipidemia presents a considerable challenge.

3 Therapeutic Approaches to Targeting Atherogenic Dyslipidemia

Lifestyle modification in terms of weight loss [25] and reduced alcohol consumption [26] can produce substantial triglyceride lowering. Fibrates are modest peroxisome proliferator-activated receptor (PPAR)-α agonists that are widely used for triglyceride lowering, with variable evidence of cardiovascular benefit in clinical outcomes trials. Early studies demonstrated benefit with gemfibrozil [27], but subsequent studies with other fibrates failed to demonstrate a clear reduction in cardiovascular events in statin-treated patients [28]. Meta-analyses of the fibrate trials reported a cardiovascular benefit that appears to be largely driven by their use in patients with baseline hypertriglyceridemia [29].

PPAR-γ agonists have been developed for glucose lowering in patients with type 2 diabetes mellitus (T2DM) because of their favorable effects on insulin sensitivity. They also modestly lower triglyceride levels [30], with favorable reductions in the triglyceride/HDL-C ratio associated with their ability to slow progression of coronary atherosclerosis in patients with T2DM [31]. Additional attempts to enhance PPAR therapeutics by increasing the potency of PPAR-α agonism or by developing dual PPAR-α/γ agonists have been disappointing, showing either no incremental benefit or toxicity [32]. Development of the selective PPAR-α modulator (SPPARM), pemafibrate, reduces triglycerides by 40–50% [33] and is currently undergoing evaluation in a large clinical outcome trial.

Niacin has a range of lipid-modifying effects, including raising HDL-C and lowering levels of both triglyceride and lipoprotein(a) [34]. However, its use has been limited by patients' difficulty tolerating high doses and a lack of cardiovascular benefit in recent trials of statin-treated patients [35, 36]. While statins modestly lower triglyceride levels, guidelines advocate intensification of their use in high-risk patients with hypertriglyceridemia. A range of novel agents are currently being developed that target factors directly implicated in metabolism of triglyceride-rich lipoproteins (apolipoprotein C3 [37], angiopoietin-like proteins 3/4 [38]) based on genetic studies that causally associated these factors with atherosclerotic cardiovascular disease.

4 Omega-3 Fatty Acids

An additional approach to lowering triglyceride levels involves the administration of the polyunsaturated omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Considerable evidence from population studies has shown an inverse association between consumption of fatty fish or omega-3 fatty acids and cardiovascular disease. Mechanistic studies have demonstrated that omega-3 fatty acids possess several functional activities that may confer a protective influence against a range of cardiovascular disease states. These include altering cell membrane function, with favorable effects on cardiac rhythm, endothelial function, and the inflammatory, oxidative, and thrombotic pathways implicated in atherosclerosis [39]. Furthermore, omega-3 fatty acids favorably modulate triglyceride-rich lipoprotein metabolism [40] (Fig. 1). These activities directly correlate with achieved EPA/DHA levels within tissue, which supports observations that the protective effect of fatty fish and omega-3 fatty acids in population studies correlates with red blood cell EPA/DHA levels [41]. In the pre-statin era, the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)-Prevenzione study demonstrated that administration of low-dose prescription

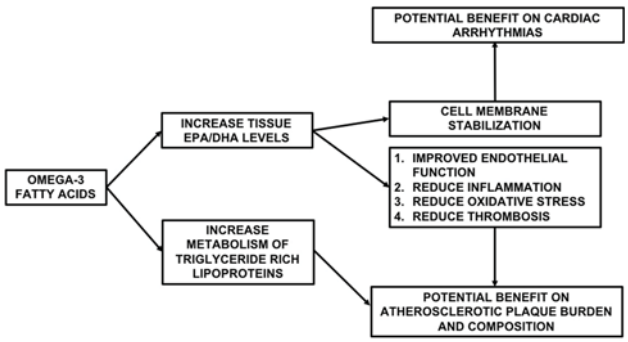


Fig. 1 Putative mechanistic effects of omega-3 fatty acids: implications for cardiovascular events. *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

omega-3 fatty acids was associated with a reduction in cardiovascular events [42]. However, with the subsequent results of the major statin trials, attention shifted to the importance of LDL-C lowering, which became the cornerstone of cardiovascular prevention guidelines.

5 Contemporary Confusion with Omega-3 Fatty Acids

Many studies have evaluated the impact of omega-3 fatty acids regarding potential mechanistic effects and their influence on clinical outcomes (Table 1). These studies have been performed in a range of contemporary settings, including their potential effect on atherosclerotic disease, heart failure, and arrhythmia and have generally produced disappointing results. This has led to meta-analyses demonstrating no cardiovascular benefits from omega-3 fatty acids [43], prompting the popular media to suggest that use of these agents for putative effects on heart disease should be abandoned.

This has been further confirmed by two recent large clinical outcomes trials that similarly produced negative findings. ASCEND (A Study of Cardiovascular Events in Diabetes) compared the effects of marine n-3 fatty acids 840 mg (EPA 460 mg and DHA 380 mg) or matching olive oil placebo daily on cardiovascular events in 15,480 patients with diabetes but without clinically manifest atherosclerotic cardiovascular disease [44]. The n-3 fatty acid group demonstrated no benefit in lipid markers and a modest increase in red blood cell EPA/DHA content of 32.5%. After a mean follow-up of 7.4 years, this was associated with no significant difference in the primary cardiovascular endpoint of nonfatal myocardial infarction, nonfatal stroke, transient ischemic attack, or vascular death (8.9 vs. 9.2% in the fatty acid and placebo groups, respectively; $p = 0.55$).

The VITAL (Vitamin D and Omega-3) trial compared the effects of the same dose of n-3 fatty acids and matching placebo in 25,871 participants in a primary prevention study of men aged ≥ 50 years and women aged ≥ 55 years [45]. Similar to ASCEND, red blood cell EPA/DHA levels increased by 54% in the fatty acid group but did not reduce the composite incidence of myocardial infarction, stroke, or cardiovascular death (3.0 vs. 3.2% in the fatty acid and placebo groups, respectively; $p = 0.24$) during a median follow-up of 5.3 years. On face value, these studies would appear to reaffirm modern data suggesting that omega-3 fatty acids are not of cardiovascular benefit. However, they add to a large body of literature in which relatively small doses of omega-3 fatty acids, resulting in—at best—modest increases in tissue levels, were administered to a wide variety of patients. These data do, however, convey an important public health message that small-dose omega-3 fatty acids that can be easily purchased over the counter should not be used primarily with the objective of preventing heart disease. However, it remains to be determined whether administration of much higher omega-3 doses with the potential to substantially raise tissue EPA/DHA levels would be protective if they were administered to patients with potentially high modifiable cardiovascular risk.

6 JELIS

The first encouraging data to emerge from a large-scale clinical trial was from JELIS (Japan EPA Lipid Intervention Study) [46]. In this open-label study, 18,645 patients with a total cholesterol level > 6.5 mmol/L were randomized to treatment with EPA 1800 mg plus statin or statin only to evaluate the impact on cardiovascular event rates. During a mean follow-up of 4.6 years, reductions in LDL-C of 25% were observed in both treatment groups, whereas the EPA group demonstrated a greater, albeit modest lowering of triglyceride levels (−9 vs. −4%; $p < 0.0001$). Despite minimal lipid differences between the groups, patients treated with EPA demonstrated a 19% reduction in the primary composite endpoint of sudden cardiac death, myocardial infarction, unstable angina, or coronary revascularization (2.8 vs. 3.5%; $p = 0.01$). Subgroup analysis revealed significant reductions in cardiovascular events with EPA only in secondary prevention patients [47]. Subsequent analyses of JELIS have reported the greatest benefit with EPA administration in patients with baseline hypertriglyceridemia [48] and in those who achieved the highest red blood cell EPA levels [49]. The modest triglyceride-lowering benefit between the groups appears to be insufficient to explain the clinical outcome benefit and suggests that an alternative mechanism was most likely responsible for the favorable results. Nevertheless, these findings prompted more widespread use of EPA for cardiovascular disease prevention in Japan.

Table 1 Features of recent large trials of omega-3 fatty acids

Trial (sample n); year	Agent + dose	Design	Inclusion criteria	Duration, years	Endpoints	Outcome
JELIS [46] (18,645); 2007	1.8 g EPA + statin	OL RCT vs. statin only; stratified primary vs. secondary	TC > 6.5 mmol/L (or LDL > 4.4 mmol/L)	4.6	Major coronary primary composite: sudden cardiac death, fatal and nonfatal MI, UA, PCI, or CABG	2.8 vs. 3.5%: HR 0.81 (95% CI 0.69–0.95), $p = 0.011$. Benefit did not extend to primary prevention cohort
ASCEND [44] (15,480); 2018	1 g (460 mg EPA, 380 mg DHA)	DB RCT vs. olive oil	Age > 40 years; DM; no CVD	7.4	Serious vascular event: nonfatal MI/stroke, TIA, vascular death. Secondary included any revascularization	8.9 vs. 9.2%: HR 0.97 (95% CI 0.87–1.08), $p = 0.55$
VITAL [45] (25,871); 2019	1 g (840 mg EPA/DHA)	DB RCT, 2×2 factorial (vitamin D vs. 'inert' placebo)	Age ≥ 50/55 years; no history of CVD	5.3	Primary MACE composite: MI, stroke, CV death. Secondary included cancer	3.1 vs. 3.2%: HR 0.97 (95% CI 0.85–1.12), $p = 0.69$
REDUCE-IT [50] (8179); 2019	4 g EPA, icosapent ethyl	DB RCT vs. mineral oil	Age ≥ 45 years; established CVD; TG 150–499; LDL 41–100; stable statin > 4 weeks	4.9	Primary composite: CV death, nonfatal MI/stroke, revascularization, UA. Secondary analysis: total events	17.2 vs. 22.0%: HR 0.75 (95% CI 0.68–0.83), $p < 0.001$ Total events: 61 vs. 89 per 1000 py: RR 0.7 (95% CI 0.62–0.78), $p < 0.001$
STRENGTH [52] (13,086); ~2020	4 g DHA/EPA, Epanova	DB RCT vs. corn oil	TG 180–500 mg/dL; HDL ≥ 42/47 mg/dL; established ASCVD; DM with RF; high-risk primary prevention	1600 primary endpoints; ~3–5	Primary composite: CV death, nonfatal MI/stroke, revascularization, hospitalization, UA. Secondary includes CV events, coronary events, time to CV death	

ASCVD atherosclerotic cardiovascular disease, *CABG* coronary artery bypass grafting, *CI* confidence interval, *CV* cardiovascular, *CVD* cardiovascular disease, *DB* double blind, *DHA* docosahexaenoic acid, *DM* diabetes mellitus, *EPA* eicosapentaenoic acid, *HDL* high-density lipoprotein, *HR* hazard ratio, *LDL* low-density lipoprotein, *MACE* major adverse cardiovascular event, *MI* myocardial infarction, *OL* open label, *PCI* percutaneous coronary intervention, *py* patient-years, *RCT* randomized controlled trial, *RF* risk factor, *RR* relative risk, *TC* total cholesterol, *TG* triglycerides, *TIA* transient ischemic attack, *UA* unstable angina

7 REDUCE-IT

REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) endeavored to test the hypothesis that administration of high-dose EPA would be cardioprotective in high vascular risk patients with hypertriglyceridemia [50]. The study recruited 8179 patients with established cardiovascular risk or the presence of diabetes and other risk factors who were treated with a statin and had a fasting triglyceride level of 150–499 mg/dL. Patients were treated with icosapent ethyl 4 g or mineral oil placebo and followed-up for a median of 4.9 years. Administration of icosapent produced a greater reduction in triglyceride levels (−18.3 vs. +2.2%; $p < 0.001$), a smaller increase in LDL-C (+3.1 vs. +10.2%; $p < 0.001$), and lower on-treatment C-reactive protein (1.8 vs. 2.8 mg/L; $p \leq 0.001$) compared with the placebo group. Unlike the previous studies, a much more robust increase of 358% in red blood cell EPA levels was observed in the icosapent group.

This increase was associated with a 25% reduction in the primary composite endpoint of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina (17.2 vs. 22.0% in the icosapent and placebo groups, respectively; $p < 0.001$). More than 12 months of treatment was required before evidence of event curve separation became apparent, and significant reductions in each component of the primary endpoint, including cardiovascular death (by 20%), were observed in the icosapent group. In contrast, all-cause mortality was not reduced, although this is not dissimilar from most contemporary large-scale cardiovascular outcomes trials. In general, icosapent was well-tolerated, although a greater rate of atrial arrhythmia (3.1 vs. 2.1%; $p = 0.004$) and a nonsignificant trend toward more serious bleeding (2.7 vs. 2.1%; $p = 0.06$) were noted in the icosapent group. A subsequent analysis of REDUCE-IT demonstrated a similarly robust decrease in the incidence of total events, highlighting the potential to substantially influence the high residual clinical risk in these patients [51].

Of particular interest was the observation of a similar degree of relative risk reduction with icosapent in patients with higher and lower triglyceride levels at study entry. To date, there is no evidence to suggest that the benefit observed in this study was derived via its triglyceride-lowering effects. The findings have not been explored in patients with different baseline EPA levels, which may provide further insights. While the mechanism for the benefit remains uncertain, the finding that the event curves did not separate for at least 12 months is consistent with several contemporary lipid-lowering trials and would suggest that it was not due to any potential antithrombotic effects of EPA. Several modest and potentially adverse biochemical effects were observed in the mineral oil placebo group. This is likely to have had a minimal impact on the event rate difference between the groups, which would have remained substantial, but it does highlight the challenge of selecting truly inert placebos in studies of omega-3 fatty acids.

8 STRENGTH

In parallel, STRENGTH (Long-Term Outcomes Study to Assess Statin Residual Risk Reduction with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia; clinicaltrials.gov NCT02104817) is evaluating the impact on cardiovascular risk of administration of high doses of combination EPA and DHA [52]. Epanova is an omega-3 carboxylic acid (EPA 75%, DHA 25%) that has undergone removal of the ethyl ester to a free fatty acid formulation during the manufacturing process. This provides an advantage in terms of removing the need for pancreatic lipase hydrolysis, required to achieve optimal intestinal absorption with omega-3 ethyl ester preparations. This permits administration irrespective of the fat content of meals. The difference in bioavailability achieves tissue EPA levels comparable to those with pure EPA formulations, with increases in trough EPA levels of up to 400% and DHA of 70%. Phase II studies in patients with hypertriglyceridemia revealed that administration of Epanova 2–4 g/day resulted in reductions in triglyceride levels of up to 30% and non-HDL-C of up to 10% [53]. In general, Epanova was well-tolerated by patients in these studies, with a higher incidence of diarrhea, nausea, and eructation being reported.

The STRENGTH study aims to determine whether administration of Epanova 4 g/day results in fewer cardiovascular events than does a matching corn oil placebo. The study has randomized 13,086 patients deemed to be at high cardiovascular risk based on a diagnosis of (1) clinically manifest atherosclerotic cardiovascular disease, (2) diabetes with an additional risk factor, or (3) requiring high-risk primary prevention and on a stable diet and statin dose for at least 4 weeks, with LDL-C < 100 mg/dL and presence of atherogenic dyslipidemia (triglyceride level ≥ 180 to < 500 mg/dL and HDL-C < 42 mg/dL for men and < 47 mg/dL for women). At least 50% of patients were required to meet the secondary prevention definition. The study has 90% power to demonstrate a 15% reduction in the primary efficacy endpoint, the time to first occurrence of the combination of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, and hospitalization for unstable angina. The trial is ongoing and will continue until 1600 primary endpoints have been adjudicated, with an anticipated median treatment duration of 3 years.

It is important to note the similarities and differences between STRENGTH and REDUCE-IT. Both studies are the first of their kind to test the impact of very high doses of omega-3 fatty acids with demonstrated ability to substantially raise tissue EPA/DHA levels. The agents differ, with icosapent being pure EPA and Epanova being a mixture of EPA and DHA. While less EPA is administered in Epanova, a similar tissue EPA level is achieved by virtue of the difference in fatty acid preparation. Icosapent is a traditional ethyl ester requiring hepatic conversion, whereas Epanova is a carboxylic acid. As a result, Epanova does not require hepatic conversion and in phase II studies achieved red blood cell EPA/DHA levels comparable to those observed with icosapent. The impact on vascular events of DHA within high-dose omega-3 fatty acids is unknown, providing an additional important feature that STRENGTH will contribute to the omega-3 fatty acid literature. Both studies required the presence of atherogenic dyslipidemia for study entry but differ in how it is defined. REDUCE-IT simply required modest hypertriglyceridemia, whereas STRENGTH requires a triglyceride level between 180 and 500 mg/dL in addition to a low HDL-C level. Whether these differences will influence the potential modifiability of cardiovascular risk in these patients is unknown. REDUCE-IT benefits from a long average treatment duration, demonstrating an increasing benefit that extends throughout the study; how this will look in STRENGTH remains to be determined. Finally, the two studies are using different placebos, although this is unlikely to produce substantial differences between the ultimate trial results.

9 Clinical Implications

It is important to note that all such pharmacological interventions for cardiovascular prevention should be applied on a background of lifestyle modifications that include changes to the diet and to exercise and smoking cessation, which have the potential to reduce triglyceride levels in their own right. The results of these studies have provided several important insights regarding targeting hypertriglyceridemia in cardiovascular prevention. The data establish that triglyceride-rich lipoproteins play an important role in the causation of atherosclerosis and drive residual risk in statin-treated patients. Observations from trials of fibrates and EPA demonstrate that targeting patients with baseline hypertriglyceridemia is beneficial and underscores the modifiability of the risk in these patients. More recent studies of omega-3 fatty acids suggest it is necessary to use high doses with the potential to profoundly elevate tissue EPA/DHA levels to observe any cardiovascular benefit with these agents. This ultimately shifts omega-3 use for cardiovascular disease prevention from over-the-counter use to high-grade pharmaceutical use. While the lack of discernible relationship between triglyceride lowering and cardiovascular benefit with these agents suggests alternative mechanisms are likely to be responsible, it continues to emphasize the importance of targeting the patient with elevated triglyceride levels in order to reduce cardiovascular risk. Other development programs are evaluating the impact on lipid levels of omega-3 phospholipid concentrate derived from krill oil as an alternative treatment in this field [TRILOGY 1 (NCT03398005) and TRILOGY 2 (NCT03361501)].

10 Conclusion

The findings of several clinical trials in recent years have suggested that residual cardiovascular risk can be modified in many statin-treated patients. The use of existing biomarkers is providing the opportunity to tailor the right therapeutic intervention to the right patient. Triglyceride levels may be one of these biomarkers, and high-dose omega-3 fatty acids present a highly effective intervention.

Compliance with Ethical Standards

Funding No sources of funding were used to conduct this review or prepare this manuscript.

Conflict of interest SJN has received research support from AstraZeneca, Amgen, Anthera, CSL Behring, Cerenis, Eli Lilly, Esperion, Resverlogix, Novartis, InfraReDx, and Sanofi-Regeneron and is a consultant for Amgen, Akcea, AstraZeneca, Boehringer Ingelheim, CSL Behring, Eli Lilly, Esperion, Kowa, Merck, Takeda, Pfizer, Sanofi-Regeneron, and Novo Nordisk. AJN and SM have no conflicts of interest that are directly relevant to the content of this article.

References

1. Cholesterol Treatment Trialists Consortium, Baigent C, Blackwell L, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376:1670–81.
2. Cannon CP, Blazing MA, Giugliano RP, et al. Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med. 2015;372:2387–97.
3. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376:1713–22.
4. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. N Engl J Med. 2018;379:2097–107.
5. Libby P. The forgotten majority: unfinished business in cardiovascular risk reduction. J Am Coll Cardiol. 2005;46:1225–8.
6. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008;118:2047–56.

7. Ference BA, Kastelein JJP, Ray KK, et al. Association of triglyceride-lowering LPL variants and LDL-C-lowering LDLR variants with risk of coronary heart disease. JAMA. 2019;321:364–73.
8. Triglyceride Coronary Disease Genetics Consortium, Emerging Risk Factors Consortium, Sarwar N, et al. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet. 2010;375:1634–9.
9. Doi H, Kugiyama K, Oka H, et al. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. Circulation. 2000;102:670–6.
10. Shin HK, Kim YK, Kim KY, Lee JH, Hong KW. Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and cytokines via lectin-like oxidized low-density lipoprotein receptor-1 activation: prevention by cilostazol. Circulation. 2004;109:1022–8.
11. Wang YI, Bettaieb A, Sun C, et al. Triglyceride-rich lipoprotein modulates endothelial vascular cell adhesion molecule (VCAM)-1 expression via differential regulation of endoplasmic reticulum stress. PLoS One. 2013;8:e78322.
12. Mansouri RM, Bauge E, Gervois P, et al. Atheroprotective effect of human apolipoprotein A5 in a mouse model of mixed dyslipidemia. Circ Res. 2008;103:450–3.
13. Jorgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjaerg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. Eur Heart J. 2013;34:1826–33.
14. Puri R, Nissen SE, Shao M, et al. Non-HDL cholesterol and triglycerides: implications for coronary atheroma progression and clinical events. Arterioscler Thromb Vasc Biol. 2016;36:2220–8.
15. Ko DT, Alter DA, Guo H, et al. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions: the CANHEART study. J Am Coll Cardiol. 2016;68:2073–83.
16. Hamer M, O'Donovan G, Stamatakis E. High-density lipoprotein cholesterol and mortality: too much of a good thing? Arterioscler Thromb Vasc Biol. 2018;38:669–72.
17. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol. 2003;23:160–7.
18. Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. Circ Res. 2004;95:764–72.
19. Nicholls SJ, Dusting GJ, Cutri B, et al. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation. 2005;111:1543–50.
20. Li D, Weng S, Yang B, et al. Inhibition of arterial thrombus formation by ApoA1 Milano. Arterioscler Thromb Vasc Biol. 1999;19:378–83.
21. Nicholls SJ, Cutri B, Worthley SG, et al. Impact of short-term administration of high-density lipoproteins and atorvastatin on atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol. 2005;25:2416–21.
22. Khera AV, Demler OV, Adelman SJ, et al. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: an analysis from the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). Circulation. 2017;135:2494–504.
23. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med. 2014;371:2383–93.
24. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation. 1990;82:495–506.
25. Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. Am J Clin Nutr. 2006;83:1025–31 **(quiz 1205)**.
26. Brinton EA. Effects of ethanol intake on lipoproteins and atherosclerosis. Curr Opin Lipidol. 2010;21:346–51.
27. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. N Engl J Med. 1999;341:410–8.
28. Keech A, Simes RJ, Barter P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005;366:1849–61.
29. Jun M, Foote C, Lv J, et al. Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. Lancet. 2010;375:1875–84.
30. Goldberg RB, Kendall DM, Deeg MA, et al. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care. 2005;28:1547–54.
31. Nissen SE, Nicholls SJ, Wolski K, et al. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. JAMA. 2008;299:1561–73.
32. Nicholls SJ, Uno K. Peroxisome proliferator-activated receptor (PPAR alpha/gamma) agonists as a potential target to reduce cardiovascular risk in diabetes. Diab Vasc Dis Res. 2012;9:89–94.
33. Arai H, Yamashita S, Yokote K, et al. Efficacy and safety of K-877, a novel selective peroxisome proliferator-activated receptor alpha modulator (SPPARMalpha), in combination with statin treatment: Two randomised, double-blind, placebo-controlled clinical trials in patients with dyslipidaemia. Atherosclerosis. 2017;261:144–52.
34. Carlson LA, Hamsten A, Asplund A. Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidaemic subjects treated with nicotinic acid. J Intern Med. 1989;226:271–6.
35. HTC Group, Landray MJ, Haynes R, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014;371:203–12.
36. A-H Investigators, Boden WE, Probstfield JL, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011;365:2255–67.
37. Gaudet D, Alexander VJ, Baker BF, et al. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. N Engl J Med. 2015;373:438–47.
38. Dewey FE, Gusarova V, Dunbar RL, et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. N Engl J Med. 2017;377:211–21.
39. Lavie CJ, Milani RV, Mehra MR, Ventura HO. Omega-3 polyunsaturated fatty acids and cardiovascular diseases. J Am Coll Cardiol. 2009;54:585–94.
40. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med. 1985;312:1210–6.
41. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis. 2007;193:1–10.
42. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999;354:447–55.
43. Aung T, Halsey J, Kromhout D, et al. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks:

meta-analysis of 10 trials involving 77917 individuals. JAMA Cardiol. 2018;3:225–34.
44. ASC Group, Bowman L, Mafham M, et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. N Engl J Med. 2018;379:1540–50.
45. Manson JE, Cook NR, Lee IM, et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. N Engl J Med. 2019;380:23–32.
46. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. 2007;369:1090–8.
47. Matsuzaki M, Yokoyama M, Saito Y, et al. Incremental effects of eicosapentaenoic acid on cardiovascular events in statin-treated patients with coronary artery disease. Circ J. 2009;73:1283–90.
48. Sasaki J, Yokoyama M, Matsuzaki M, et al. Relationship between coronary artery disease and non-HDL-C, and effect of highly purified EPA on the risk of coronary artery disease in hypercholesterolemic patients treated with statins: sub-analysis of the Japan EPA Lipid Intervention Study (JELIS). J Atheroscler Thromb. 2012;19:194–204.
49. Itakura H, Yokoyama M, Matsuzaki M, et al. Relationships between plasma fatty acid composition and coronary artery disease. J Atheroscler Thromb. 2011;18:99–107.
50. Bhatt DL, Steg PG, Miller M, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med. 2019;380:11–22.
51. Bhatt DL, Steg PG, Miller M, et al. Effects of icosapent ethyl on total ischemic events: from REDUCE-IT. J Am Coll Cardiol. 2019;73:2791–802.
52. Nicholls SJ, Lincoff AM, Bash D, et al. Assessment of omega-3 carboxylic acids in statin-treated patients with high levels of triglycerides and low levels of high-density lipoprotein cholesterol: Rationale and design of the STRENGTH trial. Clin Cardiol. 2018;41:1281–8.
53. Kastelein JJ, Maki KC, Susekov A, et al. Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: the EpanoVa fOr Lowering Very high triglyceridEs (EVOLVE) trial. J Clin Lipidol. 2014;8:94–106.

BMJ Open

Multifaceted intervention to enhance cognition in older people at risk of cognitive decline: study protocol for the Protein Omega-3 and Vitamin D Exercise Research (PONDER) study

Helen Macpherson,[1] Sarah Brownell,[1] Rachel L Duckham,[1,2] Barbara Meyer,[3] Sam Mirzaee,[4] Robin M Daly[1]

To cite: Macpherson H, Brownell S, Duckham RL, *et al*. Multifaceted intervention to enhance cognition in older people at risk of cognitive decline: study protocol for the Protein Omega-3 and Vitamin D Exercise Research (PONDER) study. *BMJ Open* 2019;**9**:e024145. doi:10.1136/bmjopen-2018-024145

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-024145).

Received 18 May 2018
Revised 19 December 2018
Accepted 12 March 2019

For numbered affiliations see end of article.

Correspondence to
Dr Helen Macpherson; helen.macpherson@deakin.edu.au

ABSTRACT

Introduction An increasing number of people are living with cognitive impairment and dementia. Current pharmacological therapies at best reduce Alzheimer's disease symptomatology but do not delay dementia onset in those at high risk. Structured exercise interventions can enhance cognition in older people; however, to produce long lasting, clinically relevant cognitive benefits, it is proposed that a multifaceted approach incorporating exercise with dietary supplements will address a wider range of mechanisms involved in cognitive decline. The Protein Omega-3 aNd vitamin D Exercise Research (PONDER) study aims to investigate the cognitive effects of a multimodal exercise programme combined with nutritional supplementation in older adults with subjective memory impairment (SMI).

Methods and analysis The PONDER study is a single-centre, 12-month, community-based, parallel group, randomised, double-blind, placebo controlled trial involving a 6-month multifaceted intervention with a further 6-month follow-up. Participants will be 148 people from Melbourne, Australia, aged 60–85 years with SMI who will be randomised (1:1 ratio) to either a 6-month supervised multimodal exercise programme combined with omega-3 fatty acid, vitamin D and protein supplementation or a stretching/flexibility exercise programme combined with placebo supplements. The primary outcome is the change in cognition after 6 months as assessed by the Trail Making Test and global cognitive function assessed from the Cogstate Computerised battery. Secondary outcomes will include memory, working memory/learning and attention/psychomotor function, the Montreal Cognitive Assessment, mood, quality of life, muscle strength, physical function, body composition, cardiovascular health and sleep quality. Cognition at 12 months will represent a secondary outcome.

Ethics and dissemination This study has been approved by the Deakin University Human Research Ethics Committee (project 2016–260). Informed consent will be obtained from all participants. The authors intend to submit the findings of the study to peer-reviewed journals or academic conferences to be published.

Trial registration number ACTRN12616001549415; Pre-results.

Strengths and limitations of this study

- This is the first randomised controlled trial to investigate the combined effects of a multimodal exercise programme with omega-3, vitamin D and protein supplementation on cognition in older adults with subjective memory impairments.
- The exercise programme is structured, supervised and delivered in a community, rather than a laboratory setting.
- The study has an additional follow-up period to enable assessment of longer term cognitive changes (over 12 months).
- It was not feasible to examine the isolated effects of the exercise programme and dietary supplement in separate study arms.

INTRODUCTION

Globally, the population is ageing, and the number of people living with dementia is set to increase by at least 50% in the next 20 years.[1] Delaying dementia onset by as little as 6 months could reduce prevalence by 6%, with a delay of 5 years predicted to decrease prevalence by 44%.[2] Therefore, strategies to reduce dementia risk factors, delay dementia onset and to slow the rate of neuropathology are urgently needed to reduce the economic and social burden of dementia and related cognitive disorders.

Alzheimer's disease (AD) is a form of dementia characterised by progressive neurodegeneration, decline in memory and other cognitive processes. Current pharmacological treatments at best reduce AD symptomatology and have not demonstrated efficacy to delay dementia onset in those at high risk of developing AD.[3] Mechanisms contributing to the neuropathology of AD are known to include neuroinflammation, oxidative stress,

mitochondrial and cerebrovascular dysfunction.[4] Interventions that target such modifiable processes, while promoting neuroplasticity and neuroprotection are essential to combat cognitive decline and AD.

Exercise, omega-3 fatty acids and vitamin D for cognitive function

Exercise is a candidate to attenuate neuropathology and enhance brain health throughout life via the regulation of growth factors and neurotrophins such as insulin-like growth-factor 1 (IGF-1), brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor that are involved in angiogenesis, synaptogenesis and neurogenesis and the modulation of systemic inflammation.[5] Exercise can positively influence cardiovascular health and insulin resistance, which are pathways relevant to cognitive decline.[6] A large body of research has demonstrated that greater levels of physical activity are associated with slower rates of cognitive decline[7] and a lower risk of dementia.[8] Furthermore, randomised controlled trials (RCTs) have shown that even acute bouts of exercise can provide small benefits to cognition.[9] Findings from recent meta-analyses have indicated that longer duration exercise interventions can enhance cognition in healthy p eople>50 years of age[10] as well as individuals at risk of or diagnosed with AD.[11]

While questions still remain regarding the optimal mode and dose of exercise required for cognitive improvements,[12] a recent systematic review and meta-analysis of 36 RCTs in community-dwelling adults, aged 50 years and above,[10] provided the recommendation that combined aerobic and resistance exercise, at a moderate to high intensity, as many days of the week as feasible, is suitable to enhance cognitive function. Exercise interventions of 4–6 months' duration that have used resistance or multimodal programmes, incorporating resistance training, have indicated that, in older people, a frequency of two sessions per week can benefit cognition.[13–15] Importantly, cognitive benefits have been observed for resistance training when compared with 'sham' exercise or stretching conditions designed not to impact heart rate or aerobic capacity.[13 14 16] The use of a control exercise condition that is matched for social interaction, including attention from the trainer delivering the programme, is necessary to account for possible benefits to cognition attributed to the social experience, rather than the exercise programme.

Accumulating evidence, largely from epidemiological studies, suggests that various dietary factors alone and in combination, particularly omega-3 fatty acids and vitamin D, are strongly linked with cognitive decline and dementia.[17–20] Polyunsaturated fatty acids (PUFAs) serve an essential role in the brain, regulating the function and structure of neurons, endothelial cells and glial cells.[21] The long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) also modify neurotransmission, reduce neuroinflammation and promote neuronal survival and neurogenesis.[21] The high intake of omega-6 fatty acids in the Western diet (ie, from vegetable oil) causes an imbalance of omega-3/6 fatty acids, resulting in lower than optimal levels of DHA and EPA.[22 23] A high intake of omega-6 fatty acids has been suggested to adversely impact cognition by depleting DHA, the omega-3 PUFA most prevalent in the brain.[24] In contrast, a low omega- 6/3 ratio has been shown to predict better cognitive function in healthy older people.[25]

Greater dietary intake of DHA (from consumption of fish) has been linked to better cognitive outcomes in older people[26] and a 36% lower risk of AD across multiple observational studies.[17] A dose–response meta-analysis showed that an increment of 100 g per week of fish intake was associated with an 11% lower risk of AD.[17] In older people with mild memory complaints (n=437) 900 mg/day of DHA supplementation for 24 weeks improved episodic memory and learning on computerised measures.[27] Of note, when compared with normative data, the improvements due to DHA supplementation on the verbal learning test approximated a 7-year improvement, compared with a 3.6-year improvement with placebo. Another study conducted in 50 older people with mild cognitive impairment found that both EPA rich (1.67 g EPA+0.16 g DHA/day) and DHA rich (1.55 g DHA+0.40 g EPA/day) supplements, taken daily for 6 months, improved depressive symptoms, compared with an omega-6 rich supplement.[28] In this study, only the DHA-rich supplement improved a single cognitive domain as well as physical health. As not all studies have yielded positive results regarding omega-3 fatty acid supplementation on cognitive function,[29–31] dose and target population may be crucial factors.

An emerging role for vitamin D in the maintenance of brain health and cognition is developing with reports that serum 25-hydroxyvitamin D (25(OH)D) levels are lower in those with impaired cognitive function and AD than healthy controls[18 32] and low vitamin D levels increase AD risk 7 years later.[33] Observational studies indicate that inadequate vitamin D intake is linked to poorer cognitive function[34] and may increase the probability of cognitive impairment by as much as 30%.[35] Relatively few RCTs have investigated the impact of vitamin D supplementation on cognition in older adults,[35] although clinical trial registries indicate a number of studies are underway. In a recent 18-week study comparing high-dose vitamin D3 (4000 IU/day) to a low dose (400 IU/day) in 82 healthy adults, the high-dose supplementation improved visuospatial memory but not other cognitive domains.[36] As even the low dose increased 25(OH)D concentrations above 75 nmol/L, this may have impacted the ability to detect cognitive changes dues to higher dose supplementation, as 75 nmol/L is the suggested optimum value to exceed to reduce cognitive and brain dysfunction.[37]

Whether there is an additive effect of different nutrients with exercise in terms of benefits to brain health is an important question. Research in rodents has indicated that DHA can enhance the effects of exercise on BDNF-related synaptic plasticity,[38] and vitamin D has

the potential to contribute to these actions through the regulation of neurotrophins BDNF, glial cell line-derived neurotrophic factor, nerve growth factor and IGF-1.[35] As a neuroprotective agent, vitamin D may target specific AD pathology, attenuating amyloid-beta (Aβ) accumulation by stimulating the phagocytosis of the Aβ peptide and inducing cytokines and macrophages to increase Aβ clearance.[35] Exercise and DHA exert potent anti-inflammatory effects[5 21] and vitamin D may further attenuate inflammation-induced degeneration of neurons by inhibiting the production of tumour necrosis factor-α, interleukin-6 and nitric oxide, preventing lipid peroxidation and reversing mitochondrial dysfunction under conditions of heightened oxidative stress.[35]

The cognitive effects of omega-3 fatty acids combined with a more complex lifestyle intervention, incorporating exercise, have recently been examined in the large 3-year RCT Multidomain Alzheimer Prevention Trial (MAPT). This study examined the impact of omega-3 fatty acid supplementation (800 mg/day DHA +250 mg/day EPA) and a multidomain intervention (cognitive training, physical activity and nutrition advice and three preventative consultations), alone or in combination, compared with placebo on cognitive function in 1680 community-dwelling, non-demented adults aged 70+ years.[39] The combination of omega-3 fatty acids and the multidomain intervention enhanced cognitive performance on a secondary measure of cognitive function and in subgroup analyses of those with an increased dementia risk score, but no benefits were found for the primary cognitive endpoints. The absence of an effect on the primary outcome for the multidomain intervention may have been influenced by the design of the exercise component that was limited to generic physical activity advice to be more active (eg, aim to walk 30 min per day) and did not include a structured programme designed to enhance physical function.

Muscle mass and strength and cognitive function

The ageing process targets multiple systems of the body, leaving older individuals vulnerable to deterioration of brain, vascular and musculoskeletal systems. A progressive loss of muscle mass, strength and function, termed as sarcopenia, occurs with ageing. After the age of 40–50 years, there is a progressive loss of muscle mass in the magnitude of 1%–2% per year.[40] Sarcopenia is a growing public health concern, increasing the risk of low trauma fracture, disability, morbidity and reducing quality of life in older people.[40]

Low muscle strength has been linked to lower global cognitive performance[41] and a higher risk of cognitive impairment.[42] Greater frailty has been associated with increased cognitive decline in patients with AD.[43] A recent investigation using data derived from the UK Biobank identified a significant relationship between grip strength and multiple cognitive domains in over 475 000 individuals from the general population.[44] While these findings emphasise the connection between muscular strength and brain health, more research is needed to determine whether increasing muscle mass and strength can enhance cognitive function. Results from the Study of Mental and Resistance Training (SMART) trial showed that strength improvements due to 6 months of high intensity resistance training were associated with improvements to cognitive function in older people with mild cognitive impairment.[45]

Combining dietary protein supplementation with resistance training exercise has been proposed as a strategy to offset muscle decline in older people.[46] A 16-week RCT conducted in older women found that increasing lean red meat intake, combined with twice weekly resistance training, increased lean tissue mass and muscle strength compared with resistance training coupled with a high carbohydrate diet.[47] A recent meta-analysis has confirmed that dietary protein can augment the effects of resistance training exercise on muscle mass and strength, although the findings indicate that resistance training is the more potent stimulus for increasing muscle strength than protein intake.[48] Protein dose may be an important consideration, with an RCT in men aged >70 years having demonstrated that providing double the recommended daily allowance (RDA) of protein (1.6 g protein/kg/day), compared with the RDA, for 10 weeks provided benefits to lean body mass and knee extension peak power.[49] These findings are consistent with the premise that older adults need a higher intake of protein to increase muscle protein synthesis.[46] With regards to the impact of combining exercise with protein on cognitive function, there is some evidence that the combination of 30 g daily protein supplementation and resistance training exercise for 6 months enhanced processing speed in frail and prefrail elderly.[50] While a consistent benefit of protein supplementation for cognition has not been established, it has been proposed that amino acids such as tyrosine and tryptophan may be capable of modulating cognitive function.[51]

Objective

It is hypothesised that a multifaceted approach combining exercise with dietary modification involving omega-3 fatty acids, vitamin D and protein may offer cognitive benefits, relative to the control condition, as they will target complementary pathways related to neuroinflammation, neuroprotection and neuroplasticity.[5 21 35]

The primary aim of this study is to investigate the efficacy of a 6-month multifaceted intervention, incorporating an exercise programme with omega-3 fatty acid, vitamin D and protein supplementation on cognitive function in older people with subjective memory complaints. The secondary aims are to: (1) determine if any beneficial effects of this intervention on cognitive function are mediated through increases in muscle strength or mass, (2) explore the impact of the intervention on other aspects of psychological and physical health and (3) evaluate the longer term residual effects (6 months after cessation of the intervention) on cognition.

METHODS/DESIGN

Study design

This is a 12-month, community-based, parallel group, randomised, double-blind, placebo-controlled trial involving a 6-month supervised and structured intervention, followed by an additional 6-month follow-up to evaluate any long-term residual effects. A total of 148 individuals with subjective memory impairment (SMI) aged 60–85 years will be randomised to: (1) a supervised multimodal exercise programme involving progressive resistance training and aerobic exercise, combined with omega-3 fatty acid, vitamin D and protein supplementation or (2) a stretching/flexibility exercise programme and placebo supplements. During the 6-month postintervention follow-up, participants will no longer be provided with the exercise programmes or supplements but will be encouraged to continue to exercise and follow a healthy diet. This study protocol is reported in accordance with the Standard Protocol Items: Recommendations for Interventional Trials statement,[52] and results will be reported in line with the Consolidated Standards of Reporting Trials (CONSORT) statement[53] and the CONSORT statement for non-pharmacological interventions.[54] The study was prospectively registered on the Australian New Zealand Clinical Trials Registry on 10 November 2016.

Participants and screening

Participants will be included into the study based on the following criteria: (1) presence of subjective memory complaints, defined as a positive response to the question 'do you feel like your memory is becoming worse?' and a score >17 on the Montreal Cognitive Assessment (MoCA), (2) community dwelling and aged 60–85 years, (3) fluent in written and spoken English and (4) with normal or corrected to normal vision and hearing.

To minimise extraneous causes of cognitive impairment, participants will be excluded based on the following: (1) diagnosis of AD or other dementia and Parkinson's disease; (2) history of brain damage including significant head trauma, stroke or serious neurological disorder, including loss of consciousness >24 hours; (3) clinical diagnosis of psychiatric disorder likely to affect cognition, including current clinical diagnosis of depression, commencement of anti-anxiety or antidepressant medication within the past 6 months, score of >5 on the short Geriatric Depression Scale; (4) or alcohol or drug dependency within the last 2 years. Exclusion criteria relevant to the study intervention, supplement and/or exercise programme, will include: (5) existing diagnosed gastrointestinal disorders likely to impact absorption of fatty acids (eg, coeliac disease); (6) known allergy to fish or any other component in the dietary supplement; (7) type two diabetes; (8) body mass index >40; (9) health conditions that contraindicate exercise or insufficient mobility to exercise; (10) participating in progressive resistance training >1 week or more >150 min per week of moderate intensity physical activity; (11) current smokers; (12) oily fish consumption (≥1/week); (13) daily use of omega-3 rich supplement >500 mg or vitamin D supplements >500 IU within the past 12 weeks; (14) use of cholinesterase inhibitors, benzodiazepines, antipsychotics or high-dose antidepressants (judged by medical advisor), use of warfarin or high dose blood thinners, resistant hypertension defined as the use of more than three antihypertensive agents and medications contraindicated for use with omega-3 fatty acids, vitamin D or protein. Any uncertainty or discrepancies about participants' clinical history will be resolved by consulting with a physician familiar with the study (investigator SM).

Participants will be recruited from within the local community in Melbourne (Australia) and surrounding areas via newspaper advertisements, community presentations, social media and community notice boards. Participants will be provided with the exercise programme free of charge as an incentive to participate in the trial.

Randomisation

Participants will be randomly allocated to one of the two parallel groups using a simple randomisation computer generated random sampling set, with an allocation ratio of 1:1. Randomisation will be stratified for gender and age, separated into three categories of 60–69.9 years, 70–79.9 and 80–85.9 years. Randomisation will be conducted by personnel not otherwise involved in the trial. Participant group allocation will be provided in an opaque envelope to the researcher involved in assigning participants to the intervention, on completion of the baseline appointment. The researcher who assigns participants to the intervention will distribute the study treatment and provide information about the assigned exercise programme. This researcher will no longer be blinded from this point.

Blinding

Both the active supplement and the placebo will be packaged in non-transparent sachets. All researchers involved in outcome assessments will be blinded to participant group allocation, with the exception of the 6-month dual-energy X-ray absorptiometry (DXA) scan, which may be conducted by an unblinded researcher due to limited qualified personnel. All DXA analyses will be carried out by a blinded researcher. During the intervention period, only unblinded researchers will be in contact with participants enrolled in the trial. In order to maintain a double-blind study design, participants will not be informed that all individuals assigned to the multimodal exercise programme will also receive the supplement drink containing omega-3, vitamin D and protein, nor that all participants assigned to the stretching/flexibility condition will also receive the placebo. The purpose of concealment will be explained when all participants have completed the trial. Exercise trainers delivering the exercise programme will not be informed that participants in the multimodal exercise programme are receiving the active supplements or those in the stretching group are receiving the placebo. All data analysis will be completed by personnel who are blinded to the intervention

assignment. Participants will be unblinded after the 12-month assessment.

The codes will only be broken in an emergency, such as a severe adverse event that requires knowledge of the treatment being taken in order to manage a participant's condition. The principal investigator and ethics committee will be informed within 24 hours of the code-break envelope being opened.

Intervention

Multimodal exercise programme plus omega-3, vitamin D and protein supplement drink

Exercise intervention: participants will take part in a 6-month group community-based programme designed by an exercise physiologist, conducted within local health and fitness centres located in Melbourne, Australia. Participants will attend 2×1 hour weekly supervised exercise sessions for 6 months at a health and fitness centre in close proximity to their home. Exercise classes will be exclusive to PONDER study participants and will not exceed 10 participants. Exercise sessions will be supervised by a dedicated, accredited exercise trainer. Each exercise session will involve a 5–10 min group warm up, 15 min of aerobic exercise followed by approximately 30–40 min of progressive resistance training concluding with a 5 min cool down. The aerobic exercises will be rotated rather than progressed. In line with current recommendations to enhance cognition,[5] the progressive resistance training will consist of two sets of 8–12 repetitions, at a weight that cannot be lifted for more than these number of repetitions, at a rating of perceived exertion of 5–8 ('hard to very-hard') on the 10-point scale.[55] This will be completed for eight resistance exercises involving dynamic concentric and eccentric contractions targeting all the major muscle groups and with an emphasis on weekly progressive overload (increments of 2%–10%). Details of the multimodal programme are shown in table 1.

Supplement: the study treatment will be a vanilla-flavoured daily supplement drink containing 5 g of omega-3 powder (18% EPA, 12% DHA equivalent to 900 mg EPA and 600 mg DHA), vitamin D3 1000 IU and 25 g of whey protein concentrate (WPC) 80. The total of 1.5 g omega-3 fatty acids is a physiologically relevant dose (equal to half portion of farmed Atlantic salmon or a full portion of fresh or tinned wild Atlantic salmon).[56] The aim of 1000 IU/day vitamin D3 treatment is to raise serum 25(OH)D concentrations to at least 50–60 nmol/L, in line with current Australian recommendations.[57] When combined with resistance training, 20 g of whey protein isolate has been demonstrated to increase muscle protein synthesis in older people[58]; therefore, 25 g of WPC was selected to provide a comparable dose of protein. Each drink will provide approximately 590 kJ of energy, 2 g of lactose, 6 g of fat and 20 g of protein. The supplement will be provided in powder form to be mixed with 200 mL water. The treatments have been developed by Omniblend Australia. Participants will be instructed to

Table 1 Details of the exercise programmes

	Multimodal exercise
Frequency	2× per week.
Intensity	RPE rating of 5–8 (hard to very hard).
Duration	60min.
Warm up	5min group-based warm up including balance and mobility exercises.
Aerobic	15 min aerobic training rotating exercises: treadmill, bike, stepper, rower, X-trainer/elliptical trainer and so on.
Resistance	30–40 min resistance training: eight exercises (six resistance, two core), two sets, 8–12 reps targeting all the major muscle groups and with an emphasis on weekly progressive overload (increments of 2%–10 %). Resistance exercises may include: squats / leg press, lat pulldown, calf raises, chest press/seated row, modified plank and so on.
Cool down	5–10min cool down: relaxation type exercises incorporating stretching.
	Stretching and flexibility.
Frequency	2× per week.
Intensity	Light
Duration	50–60 min.
Warm up	5 min light walking/marching, range of motion exercises.
Aerobic	Nil.
Resistance	Nil.
Flexibility	35–40 min variety of light stretching/flexibility exercise and light resistance band exercises that will not increase strength.
Cool down	5–10 min cool down: relaxation type exercises.

RPE, rating of perceived exertion.

consume the supplement immediately before breakfast, except on days involving the exercise programme, when they will be requested to consume the supplement 1–2 hours after completing the session. To reduce the likelihood of any gastrointestinal side effects, participants will be instructed to consume only half the supplement dose for the first 7 days of the intervention.

Control group (stretching/flexibility programme+placebo drink)

Exercise: participants will complete a 6-month group community-based stretching/flexibility programme designed by an exercise physiologist within local health and fitness centres. Participants will attend 2×1 hour weekly supervised exercise sessions for 6 months. Groups will be exclusive to study participants. The exercise programme will involve stretching and is designed not to influence heart rate, strength or aerobic capacity but to provide a similar level of social contact and attention from a dedicated exercise trainer at each centre. Details of the stretching/flexibility programme are shown in table 1.

Supplement: the placebo drink will be vanilla flavoured and matched for appearance and caloric content and will contain 16 g whole milk powder and 15 g maltodextrin. Each drink will provide approximately 590 kJ of energy.

Compliance

Compliance to the exercise programmes will be assessed using attendance records and for the multimodal group, monthly exercise cards collected from the exercise trainers. Each exercise card contains information regarding sessions attended, exercises completed, set, repetitions, time and training intensity. A compliance calendar showing each month will be provided to assess supplement compliance. Participants will mark off each day the supplement is consumed and record any reasons for variations (ie, missed supplement or taken later in the day). Participants who are non-compliant with the intervention will still be requested to attend all follow-up appointments.

Adverse events

Any adverse events potentially or confirmed to be associated, with the exercise programme will be recorded on the exercise cards provided to the exercise trainers. The supplement calendar will be used to record adverse events potentially due to the supplement. Any health-related unfavourable or unintended medical occurrence (eg, sign, symptom, syndrome and illness), deemed to be relevant to the study, that develops or worsens during the trial will be classed as an adverse event. All adverse events will be assessed for seriousness, severity, causality and expected outcome by the researchers. There will be no formal data monitoring committee, due to the size of the study and the single site; however, all data will be reviewed by the research team at regular intervals throughout the study.

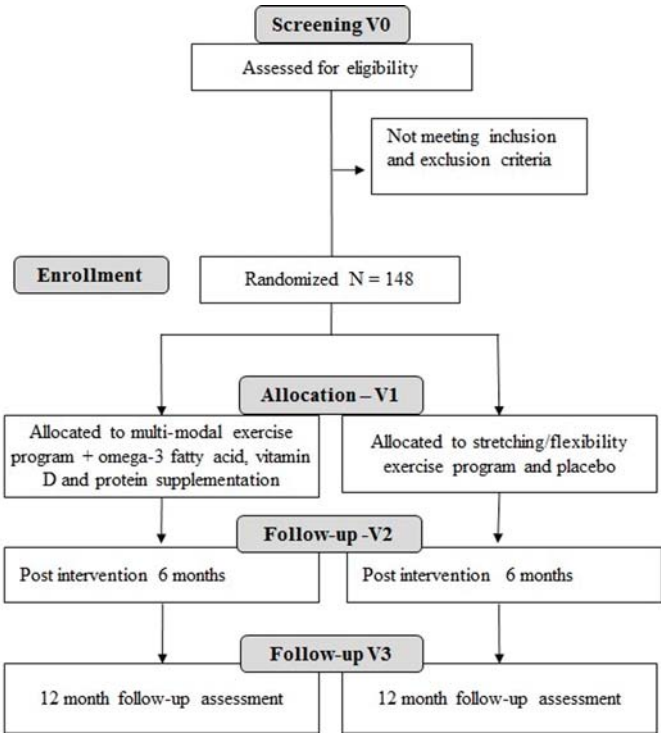


Figure 1 Flow diagram: participant recruitment and screening flow chart.

Sample size calculation

To date, no studies have investigated the cognitive effects of the specific intervention combination examined in the current study. For this reason, the anticipated effect size is largely based on findings derived from the exercise literature. Exercise exerts an average 0.5 SD effect size (equating to a medium effect size) on executive function.[5] A recent meta-analysis has indicated that multimodal exercise interventions of at least 4 weeks' duration have a positive effect on cognition in the order of a 0.33 effect size, in people over the age of 50 years.[10] Based on these figures, we anticipate at least a medium effect size (f=0.25). Allowing for 15% attrition rate, a sample of 148 participants, with 74 individuals assigned to each study arm, will provide a power level of 0.8 at the 5% probability level.

Procedure

Participants will attend a total of four visits to Deakin University in Melbourne to undergo study assessments (see figure 1). All participants will be requested to abstain from alcohol for 24 hours prior to study visits. No caffeine is to be consumed 2 hours prior to study visits, and participants will consume the same food and drink at the baseline (V1) and 6-month (V2) visits. Baseline and 6-month visits will be held at the approximate same time of day (within 2 hours).

- *Prescreening:* all participants will undergo telephone screening.
- *Screening (V0):* potential participants who meet the prescreening study criteria will attend a face-to-face session with a researcher to provide written informed

consent and undergo screening for inclusion/exclusion criteria. Eligible individuals will undergo familiarisation of core study measures and will be provided with a physical activity monitor and pathology collection requests to complete blood collection prior to V1. Participants requiring medical approval must obtain this prior to baseline.

- *Baseline (V1):* to be conducted within 4 weeks of the V0 screening visit. During V1, cognitive assessments will be carried out first, followed by cardiovascular measures, anthropometry, DXA, functional measures and questionnaires. On completion of V1, participants will be allocated to either the intervention group or control group and provided with the supplement and details of the relevant gym programme. Participants are requested not to commence additional omega-3 or vitamin supplementation during the trial or to take part in more than one additional structured exercise class per week.
- *Intervention period:* an unblinded study member will remain in contact with participants via telephone and face-to-face visits at the gyms and will collect exercise cards, including adverse event logs and supplement compliance logs. Possible adverse events, gym non-attendance and supplement non-compliance will be followed up by telephone by an unblinded researcher.
- *6 month follow-up (V2):* participants will repeat physical activity monitoring and blood collection within the week prior to V2. All measures from baseline will be repeated along with the MaCA.
- *12-month follow-up (V3):* due to funding constraints, only cognitive assessments including the Cogstate, Trail Making Test and MoCA and study questionnaires will be completed at V3.

Primary outcome

Change in global cognitive function derived from the CogState computerised battery and the Trail Making Test after 6 months.

Secondary outcomes

Changes in cognitive function for the six individual measures and the composite scores of working memory/learning and attention/psychomotor function assessed using the CogState battery, the Trail Making Test, the MoCA after 6 months and 12 months, and changes in mood, health-related quality of life, strength, physical function, body composition, cardiovascular health and sleep quality after 6 months. A list of outcomes and time points are shown in table 2.

Screening measures

The self-administered 15-item Short Form Geriatric Depression Scale (GDS)[59] will be used to screen for the presence of depression at V0. Scores greater than 5 potentially indicate depression and those who score >5 will not be eligible for the study. The interviewer administered MoCA[60] will be used to screen for the possible presence of dementia. The MoCA is scored out of 30 and assesses short-term memory, visuospatial ability, executive function, verbal fluency, attention and working memory and orientation to time and place and is summed to create a global measure of cognition. Participants require a score >17 to be eligible for the study. The MoCA has been shown to have 90% sensitivity and 87% specificity for detecting MCI (n=94) and 100% and 87%, respectively, for detecting early AD.[60] The MOCA will be used for screening purposes at V0 and repeated as a secondary outcome after 6 months (V2) and 12 months (V3).

Primary outcome measures

Cognitive assessments

The Cogstate Computerised Battery (www.cogstate.com) will be used to assess cognition for six cognitive tasks, which will form the basis for two composite scores (working memory/learning and attention/psychomotor function) and a measure of global cognitive function, which will be the primary outcome. The individual cognitive tasks include the *Detection Task* (reaction time task measuring psychomotor function), *the Identification Task* (choice reaction time task measuring visual attention), the *One Card Learning Task* (visual recognition memory and attention) and the *One-Back Task* (working memory and attention). For all tasks, speed (reaction time in milliseconds) and accuracy (number of correct responses made) will be recorded. The fifth task is the *Groton Maze Task*, which assesses executive function and is calculated based on the number of errors. The final task is the *International Shopping List* (verbal learning and memory), which is scored using accuracy, calculated by summing correct responses. Individual raw scores for each task will be transformed into a z-score using the mean and SD of the total sample in the study. Composite scores for working memory/learning will be calculated by averaging the standardised scores (z-score) for the One Card Learning and One-Back Tasks, and the attention/psychomotor function composite will be calculated by averaging the z-scores for the Detection and Identification Tasks. Global cognitive function will be derived as the average z-scores from all six tests, which higher scores indicating better performance. The CogState computerised tasks are specifically designed for repeated administration over short time periods and have been validated in older adults.[61–63] Practice or fatigue effects are minimal for these tasks, making them suitable for detecting subtle cognitive changes.[61–63] To further minimise practice effects, the entire battery will be practised at V0.

The Trail Making Test[64 65] Part A (visuoperceptual ability) involves drawing lines to connect numbered dots in ascending numerical order as quickly as possible while still maintaining accuracy. Part B (working memory and task-switching ability) involves connecting dots in ascending order, alternating between numbers and letters as quickly as possible. The score is the number of seconds taken to complete each part of the test. The score from Part B minus Part A provides an indication of executive

Table 2 Outcome measures and relevant data collection time points

Outcome measures	Data collection method	Visit 0: practice	Visit 1: baseline	Visit 2: 6 month	Visit 3: 12 month
Primary outcome					
Cognitive function	Cogstate Computerised Battery	Practice	X	X	X
	Trail Making Test	Practice	X	X	X
Additional outcomes *Cognitive function*	Montreal Cognitive Assessment	X		X	X
	NART-R IQ	X			
	Cognitive Function Instrument		X	X	X
Muscle strength	3RM Knee extension	Practice	X	X	
	Hand grip strength		X	X	
Physical function	Four Metre Walk Test		X	X	
	Four-Square Step Test		X	X	
	30 s Sit-to-Stand Test		X	X	
	Timed Up and Go Test		X	X	
Mood	Short Geriatric Depression Scale	X			
	Depression Anxiety Stress Scale		X	X	X
Quality of life	Short-form (SF)-36 version 2		X	X	X
Sleep	Pittsburg Sleep Quality Index		X	X	X
Personality	Short NEO-Personality Inventory	X			
Demographics/lifestyle	Lifestyle Questionnaire		X	X	X
Anthropometry	Body mass index		X	X	
	Hip, waist and neck circumference		X	X	
Body composition	DXA (total body and regional)		X	X	
Cardiovascular function	Resting blood pressure				
	Central arterial pressure, pulse wave velocity and arterial stiffness		X	X	
Diet	ASA-24 Dietary Assessment Tool		X	X	X
	PUFA Questionnaire		X	X	
Habitual physical activity	CHAMPS Survey		X	X	X
	7-day physical activity (Actigraph)		X	X	X
Biomarkers	Cholesterol and HsCRP		X	X	
	Omega-3 fatty acids and vitamin D		X	X	
	Apolipoprotein E e4 status		X		

3RM, three repetition maximum; ASA-24, Automated Self-Administered 24 hours; CHAMPS, Community Healthy Activities Model Program for Seniors; DXA, dual-energy X-ray absorptiometry; HsCRP, high-sensitity C reactive protein; NART-R IQ, National Adult Reading Test Revised IQ; PUFA, polyunsaturated fatty acids.

function[65] and will be considered the primary outcome generated from the Trail Making test. The Trail Making Test has test–retest reliability ranging from 0.74 to 0.85.[66] To minimise practise effects, the Trail Making Test will be practised at V0.

Secondary outcome measures

Mood, quality of life and sleep

The 21-item Depression, Anxiety and Stress Scale (DASS)[67] will be used to assess mood. The DASS consists of three subfactors that yield subscores for each domain: depression, anxiety and stress. Higher total scores indicate a higher degree of mood dysfunction.

The self-administered Short Form 36 version 2 (SF-36 v2) questionnaire will be used to measure health-related quality of life.[68] The SF-36 covers the domains of physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health. The current study will report Australian norm-based scores using previously published guidelines[69] to provide each domain score a standardised mean of 50

and a SD of 10. Separate quality of life summary scores will be calculated for the physical component and the mental component.

The self-administered 19-item Pittsburgh Sleep Quality Index (PSQI) will be used to measure sleep quality and disturbance over a 4-week period.[70] The scale is composed of items that generate seven subscores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medications and daytime dysfunction. Both the total score and subscores will be outcomes for this measure.

Muscle strength

Lower body muscle strength will be determined by the Keiser bilateral knee extension machine (Keiser Corporation, Fresno, California, USA), using a standard three repetition maximum protocol (see ref 71). Participants will warm up with 8 repetitions at 3 kg. The weight will be increased progressively to participant's estimated three repetition maximum (kg). After a 2 min rest, resistance will be increased (or decreased) until only three repetitions in good form can be completed.

Bilateral maximal isometric grip strength will be measured using a hand-held dynamometer (Jamar dynamometer, Asimov Engineering Co, Los Angeles, California, USA). In a seated position, with elbow flexed at 90°, forearm neutral and hand slightly extended, participants will be instructed to squeeze the hand grip as tightly as possible and release for three trials with a 1 min rest between trials. After a practise trial, the maximal grip strength (kg) of each hand will be recorded. The test is performed by both the dominant and non-dominant limbs.

Physical function

All functional tests will be preceded by a practise trial. The 4 m walk test will be used to assess gait speed. Participants walk in a straight line at their normal walking speed across four metres with speed recorded using timing gates (Swift Speedlink Performance Equipment systems). The outcome will be the fastest time measured in seconds (to the nearest millisecond) from two trials.

The Four-Square Step Test (FSST) will be used to assess dynamic standing balance and stepping speed in four different directions.[72] The FSST is completed first in a clockwise and then counter-clockwise direction. Participants will complete two test trials, and the outcome will be the fastest time (in seconds) taken to complete the sequence.

The Timed Up and Go Test measures dynamic balance during three commonly performed functional activities: standing up from and sitting down in a chair, walking and turning.[73] Participants are required to stand up from a chair, walk as quickly as possible around a cone (marking 3 m), turn and walk back to the chair and sit down. The outcome will be the time (in seconds) to complete the sequence.

The 30 s Sit-to-Stand Test[74] will be used to assess lower extremity muscle strength and power. Starting from a seated position in a chair, chair measuring 45 cm from the ground, with arms folded across the chest, participants stand fully upright and then return to the seated position as many times as possible in 30 s. The outcome is the number of sit-to-stands completed in 30 s.

Anthropometrics and body composition

Height will be measured in centimetres using a stadiometer fixed to the wall, and weight will be measured in kilograms using calibrated scales with standardised protocols. Body mass index (BMI) will be calculated as weight (kg)/height (m^2). Waist and hip circumference will be measured in centimetres using the WHO protocol.[75] Waist-to-hip ratio will be calculated by dividing the waist circumference measurement by the hip circumference. Neck circumference will be measured around the midpoint of the participant's neck, between the midcervical spine and mid anterior neck. All measures will be taken twice and the average value used as the outcome.

Dual-energy X-ray Absorptiometry (DXA) will be used to assess body composition (fat, lean and bone) (Lunar iDXA, version Encore 16.2, GE Healthcare Medicine, Wisconsin, USA). Total body lean mass, fat mass and percentage body fat will be determined from the total a regional (arm and legs) body. Standardised procedures (2015 ISCD official positions) for participant positioning and scan analysis will be used for all scans (https://www.iscd.org/official-positions/2015-iscd-official-positions-adult/). During each visit participants will be scanned in a hospital gown and instructed to remove all metal objects. All scans for all participants will be performed by the same operator on the same scanner following daily quality assurance and control monitoring.

Cardiovascular health

Seated blood pressure will be assessed using an ambulatory blood pressure monitor. The blood pressure cuff will be fixed to the left arm following a 5 min period of seated rest. Four consecutive measurements will be taken at 1 min intervals and the average of the final three readings will be used as the outcome.

The SphygmoCor XCEL (AtCor Medical PTY) will be used to measure central arterial pressure waveform, pulse wave velocity (PWV) and arterial stiffness. Pulse wave analysis (PWA) will be measured after 5 min of quiet supine rest with the cuff attached to the left arm held in a straight position. Measures will be taken twice and the average value used at the outcome. To conduct carotid-femoral PWV, a cuff will be placed around the femoral artery to capture the femoral waveform, and a tonometer will capture the carotid waveform. The distance between the carotid and femoral arteries will be measured, and the velocity automatically calculated by dividing the distance by the pulse transit time. Measures will be taken twice, with a third measure completed if the difference between

the PWV readings is >0.5 m/s. The average value will be used at the outcome.

Demographic variables and potential covariates

Demographic and lifestyle information will be collected on participant's ethnic and educational background, employment history/status, medical history, history of falls and fractures, family history of osteoporosis, current medication and dietary supplement use, alcohol intake, weekly television viewing and sitting time and sun exposure habits.

Cognitive and psychological factors

The National Adult Reading Test-Revised (NART-R) will be used to estimate IQ.[76] The NART-R is suitable to assess premorbid intellectual functioning in the presence of cognitive impairment.[77] The number of errors in pronunciation will be converted to an estimate of Full-Scale IQ score. The Cognitive Function Instrument[78] is a self-report measure of 14 items that probe experiences of memory decline over the past year. This measure will be used to provide greater detail regarding subjective cognitive complaints and has been demonstrated to correlate with objective cognitive decline.[79] The short 60-item NEO Personality Inventory will be used to assess the five major personality domains of extraversion, agreeableness, conscientiousness, neuroticism and openness to experience[80] as personality characteristics may contribute to subjective cognitive complaints.[81]

Blood biomarkers

Within 7 days prior to baseline (V1) and 6 months (V2), participants will attend a commercial pathology clinic collection centre where a fasted, morning (08:00–10:00) venous blood sample will be collected. Blood samples will be separated into serum/plasma and red blood cells by low speed centrifugation (3000 rpm, 4°C for 10 min). The following samples will be sent to a central pathology laboratory accredited by the National Association of Testing Authorities Royal College of Pathologists Australasia: serum total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides will be analysed using an enzymatic colorimetric method (Roche Diagnostics, Mannheim). Low-density lipoprotein (LDL)-cholesterol will be calculated using Friedewald's formula. Serum creatinine, urea, albumin, calcium, phosphorus, sodium, potassium and phosphorus will be analysed using standardised techniques. Additional packed red blood cells, plasma and serum samples will be stored until analysed. Samples will be stored in a −70°C freezer prior to being transferred to Deakin University, where samples will be stored at –80°C until the study is complete. Erythrocyte levels of omega-3 PUFAs will be extracted based on LePage and Roy method.[82] The extracted fatty acids will be separated by gas chromatography, identified by the comparison with known fatty acid standards and quantified by comparison to the internal standard (21:0). Serum 25(OH)D will be used to determine changes in vitamin D levels due to the supplement.

Apolipoprotein E (ApoE) status

ApoE is involved in lipid homeostasis, particularly in the determination of the levels of LDL and HDL cholesterol, which are directly and inversely correlated with risk for cardiovascular disease respectively.[83] Carriers of the ApoE e4 allele have increased coronary risk,[84] and e4 carriers also have a higher risk of developing late onset AD.[85] Although e4 carriers may experience a similar benefit from lifestyle based interventions, their rate of cognitive change may be greater than in non-carriers.[86] ApoE status will be assessed at baseline (V1), DNA will be extracted from venous whole blood samples using commercially available kits.

Physical activity

The Community Healthy Activities Model Program for Seniors (CHAMPS) physical activity questionnaire will be used to calculate total leisure and recreational physical activity time. This measure has been designed for use in older adults and is reliable, valid and sensitive to change.[87]Individuals will document their weekly frequency and duration of participation in a 'typical week' of the preceding 4 weeks. Outcomes will be hours per week, and estimated kilojoules per week, spent in moderate to high intensity activities.

To obtain an objective measure of physical activity, participants will be provided with an Actigraph monitor to wear for a period of up to 7 days on three occasions. The Actigraph is a small lightweight monitor that will be worn on the right hip to measure quantity and intensity of physical activity. Monitors will be removed for sleep. Participants will complete a log of their activity including sleep hours to aid in interpretation of this data. Physical activity information will be collected for the 7 days prior to V1, V2 and V3 assessments. Time spent in sedentary, light, moderate and vigorous activity will be calculated.

Diet

The Automated Self-Administered 24 hours (ASA24) Australia Dietary Assessment Tool (https://epi.grants.cancer.gov/asa24/) developed by the National Cancer Institute will be used as a 24-hour dietary recall. The ASA24 is completed online; however, it will be conducted over the phone with a researcher if participants do not have computer and internet access. The ASA24 allows respondents to report foods and drinks and amounts consumed by browsing a food category or searching from a list of food and drink terms derived from the 2007–2008 National Health and Nutrition Examination Survey. The ASA-24 provides a breakdown of macronutrients and energy, vitamins, minerals, carotenoids, fats, cholesterol and fatty acids.

A validated online questionnaire will be used to assess dietary PUFA intake. The PUFA questionnaire is a simple non-invasive method that estimates the intakes of omega-3

and omega-6 PUFAs in a healthy adult population.[88] The questionnaire includes main food sources of α-linolenic acid as well as omega-6 PUFA, linoleic acid (LA) and arachidonic acid (AA). The PUFA questionnaire consists of 38 questions relating to the usual dietary habits related to PUFA intake over 3 months. The questions pertain to foods, such as fish, meat and eggs for omega-3 PUFA, and vegetable oils and fats for omega-6 PUFA. The questionnaire covers fish oil supplement consumption and products with added omega-3 such as bread, eggs and milk.

Statistical analysis

Data will be analysed using SPSS V.25 and STATA V.15 applying standard statistical thresholds ($p<0.05$), corrected for multiple comparisons where appropriate. There will be no interim data analysis. All data will be checked for normality prior to analysis. Data will be analysed based on an intention-to-treat approach using linear mixed models with random effects. Changes in the primary outcomes (CogState global cognitive function and Trail making test of executive function), and all secondary outcomes will be expressed as the absolute or percentage difference for the change between the intervention group and control group after 6 months or 12 months, as appropriate. For the cognitive outcomes, the results will be analysed adjusted for age, gender and years of education (model 1) as well as baseline MoCA score and ApoE status (model 2). Per-protocol analysis will be used to determine the effectiveness of the intervention among participants who are considered to be compliant with the intervention. Participants who attended 67% (two-thirds) of exercise sessions and consumed 80% of the allocated supplement will be classified as adherent for the purpose of completer analysis. All data will be presented as mean±SD or 95% CIs.

Ethical approval, data management and dissemination plan

The current protocol is based on version 4, April 2017. The first participants were enrolled into the study on 4 April 2017. In line with the ethical approval for this study, all data will be de-identified and stored in a locked filing cabinet or on a secure data drive at Deakin University. Only members of the research team will have access to the data. Only study investigators will have access to the final data set. Any changes to the trial protocol will be reported to Deakin University Human Research Ethics Committee and updated on the clinical trial registry. On completion of the trial, participants will receive a report outlining major study findings. They may also request pathology results. Study investigators will disseminate group results to the public in the form of journal articles, conference presentations, seminars and community presentations.

Patient and public involvement

Participants were not explicitly involved in the development of the trial. Feedback will be sought from all participants at the end of the study to assess burden of intervention and to help develop future trials. Participants will receive individual result summaries of key measures and a summary of the overall study findings.

DISCUSSION

Given that dementia is among one of the major causes of disability and death worldwide,[89] interventions that prevent cognitive decline and delay the onset of dementia and related disorders are urgently needed. This study will be the first to evaluate whether a multifaceted intervention comprising of exercise combined with omega-3, vitamin D and protein supplementation can improve cognitive function in older adults at risk of cognitive decline and provide information about potential underlying mechanisms. This study will evaluate the longer-term cognitive impact of the multifaceted intervention.

Age-related impairments to cognitive and physical function are multifactorial; therefore, multidomain interventions that target multiple risk factors are needed to address a wider range of mechanisms involved in cognitive and functional decline. Promising results have been obtained from the large-scale, 2-year Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) trial, which examined the impact of a multidomain intervention on cognitive function in 1260 older adults aged 60–77 years, selected on the basis of elevated risk for dementia.[90] The study intervention included four components including personalised and group dietary recommendations, an individually tailored exercise programme targeting progressive muscle strength and aerobic exercise, group and web-based cognitive training and management of metabolic and vascular risk factors compared with regular health advice. The results of this study demonstrated a significant benefit of the multidomain intervention to a composite cognitive score, a processing speed and executive function composite score, with improved or maintained cognitive function from 25% to 150% better than the control group. The only cognitive domain not to demonstrate an improvement was memory. These findings are important as they suggest that making multiple lifestyle modifications can, in turn, modify cognition.

While the results of the MAPT did not show benefits of a combined multidomain and omega-3 fatty acid intervention to the primary cognitive outcomes, some cognitive improvements were seen for secondary outcomes and subgroup analyses. Currently, the SYNchronizing Exercises, Remedies in Gait and Cognition (SYNERGIC) trial is underway to investigate whether a 20-week multimodal intervention combining aerobic and resistance training exercise, with or without cognitive training or vitamin D supplementation, can improve cognition and reduce falls in 200 adults with mild cognitive impairment.[91] Similar to the current study, the SYNERGIC trial is focused on interventions that simultaneously target cognitive health and physical function. A common element of these studies is that they have incorporated cognitive training in the

multidomain interventions. Yet the addition of cognitive training to resistance training was less effective than exercise alone for cognitive function in the SMART trial.[13] The premise of 'overdosing' by including too many lifestyle intervention components has been suggested as a factor that may have contributed to these findings.[92] In the current study, we have opted to limit the intervention to exercise and dietary supplementation and not include cognitive training. The dietary component of the PONDER study is also restricted to a supplement, rather than individualised dietary counselling in order to simplify the lifestyle changes required to be undertaken by participants.

Similar to the FINGER study,[90] a potential limitation of the current study is that it was not feasible to examine the isolated effects of the exercise programme and dietary supplement in separate study arms. However, the study aim is to evaluate the combined impact of the exercise programme and dietary supplement on cognitive function. The duration of the PONDER intervention is shorter than the larger FINGER and MAPT trials; however, the SMART trial demonstrated cognitive benefits after only 6 months of resistance training[13] indicating our study intervention duration should be sufficient to modify cognitive function. Furthermore, our study includes an additional 12-month follow-up to capture residual effects of the intervention. Finally, our study criteria does not exclude individuals with mild cognitive impairment, only those with dementia thus may include participants in the early stages of cognitive decline or preclinical dementia.

To summarise, this is the first RCT to investigate the combined effects of a multimodal exercise programme, omega-3 fatty acid, vitamin D and protein supplementation on cognitive function in older people with SMI. This study will contribute to a growing research field which assesses the effectiveness of multidomain, lifestyle-based, interventions for cognitive decline in the elderly.

Author affiliations
[1]Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Geelong, Victoria, Australia
[2]Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne and Western Health, St Albans, Victoria, UK
[3]Faculty of Science Medicine and Health, University of Wollongong, Wollongong, New South Wales, Australia
[4]Monash Cardiovascular Research Centre, Monash HEART, Monash University, Melbourne, Australia

Contributors All listed authors fulfil the ICMJE guidelines for authorship made substantial contributions to conception and design of the trial. The exercise programme was developed by SB and RLD. SM is the medical overseer of the trial. HM drafted the article, and all authors revised the manuscript critically for important intellectual content and provided final approval of the version to be published.

Funding This work was supported by National Health and Medical Research Council - Australian Research Council (NHMRC-ARC) grant number (APP1097696). In kind support was provided by Omniblend Australia for supplements. The funding body has had no role in study design; data collection, management and will have no role in analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical approval was obtained from the Deakin University Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Prince M, Bryce R, Albanese E, *et al*. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement* 2013;9:63–75.
2. AIHW, Australia's health 2013. *The thirteenth biennial health report of the Australian Institute of Health and Welfare (AIHW)*. Canberra, 2012.
3. Livingston G, Sommerlad A, Orgeta V, *et al*. Dementia prevention, intervention, and care. *The Lancet* 2017;390:2673–734.
4. Jack CR, Knopman DS, Jagust WJ, *et al*. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 2010;9:119–28.
5. Chang YK, Pan CY, Chen FT, *et al*. Effect of resistance-exercise training on cognitive function in healthy older adults: a review. *J Aging Phys Act* 2012;20:497–517.
6. Kennedy G, Hardman RJ, Macpherson H, *et al*. How Does Exercise Reduce the Rate of Age-Associated Cognitive Decline? A Review of Potential Mechanisms. *J Alzheimers Dis* 2017;55:1–18.
7. Sofi F, Valecchi D, Bacci D, *et al*. Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. *J Intern Med* 2011;269:107–17.
8. de Bruijn RF, Schrijvers EM, de Groot KA, *et al*. The association between physical activity and dementia in an elderly population: the Rotterdam Study. *Eur J Epidemiol* 2013;28:277–83.
9. Chang YK, Labban JD, Gapin JI, *et al*. The effects of acute exercise on cognitive performance: a meta-analysis. *Brain Res* 2012;1453:87–101.
10. Northey JM, Cherbuin N, Pumpa KL, *et al*. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. *Br J Sports Med* 2018;52:154–60.
11. Panza GA, Taylor BA, MacDonald HV, *et al*. Can Exercise Improve Cognitive Symptoms of Alzheimer's Disease? *J Am Geriatr Soc* 2018;66:487–95.
12. Barha CK, Galea LA, Nagamatsu LS, *et al*. Personalising exercise recommendations for brain health: considerations and future directions. *Br J Sports Med* 2017;51:636–9.
13. Fiatarone Singh MA, Fiatarone Singh MA, Gates N, *et al*. The Study of Mental and Resistance Training (SMART) Study-Resistance Training and/or Cognitive Training in Mild Cognitive Impairment. *JAMDA* 2014;15:873–80.
14. Nagamatsu LS, Chan A, Davis JC, *et al*. Physical activity improves verbal and spatial memory in older adults with probable mild cognitive impairment: a 6-month randomized controlled trial. *J Aging Res* 2013;2013:1–10.
15. Vaughan S, Wallis M, Polit D, *et al*. The effects of multimodal exercise on cognitive and physical functioning and brain-derived neurotrophic factor in older women: a randomised controlled trial. *Age Ageing* 2014;43:623–9.
16. Kelly ME, Loughrey D, Lawlor BA, *et al*. The impact of exercise on the cognitive functioning of healthy older adults: a systematic review and meta-analysis. *Ageing Res Rev* 2014;16:12–31.
17. Wu S, Ding Y, Wu F, *et al*. Omega-3 fatty acids intake and risks of dementia and Alzheimer's disease: a meta-analysis. *Neurosci Biobehav Rev* 2015;48:1–9.
18. Goodwill AM, Szoeke C. A Systematic Review *and Meta-Analysis of The Effect of Low Vitamin D on Cognition*. *J Am Geriatr Soc* 2017;65:2161–8.
19. Jayedi A, Rashidy-Pour A, Shab-Bidar S. Vitamin d status and risk of dementia and alzheimer's disease: A meta-analysis of dose-response. *Nutr Neurosci* 2018;11:1–10.
20. Patrick RP, Ames BN. Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior. *Faseb J* 2015;29:2207–22.
21. Bazinet RP, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci* 2014;15:771–85.
22. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991;54:438–63.

23. Meyer BJ. Australians are not meeting the recommended intakes for omega-3 long chain polyunsaturated fatty acids: Results of an analysis from the 2011-2012 national nutrition and physical activity survey. *Nutrients* 2016;8:111.
24. Sheppard KW, Cheatham CL. Omega-6 to omega-3 fatty acid ratio and higher-order cognitive functions in 7- to 9-y-olds: a cross-sectional study. *Am J Clin Nutr* 2013;98:659–67.
25. Andruchow ND, Konishi K, Shatenstein B, *et al*. A lower ratio of omega-6 to omega-3 fatty acids predicts better hippocampus-dependent spatial memory and cognitive status in older adults. *Neuropsychology* 2017;31:724–34.
26. Fotuhi M, Mohassel P, Yaffe K. Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association. *Nat Rev Neurol* 2009;5:140–52.
27. Yurko-Mauro K, McCarthy D, Rom D, *et al*. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. *Alzheimers Dement* 2010;6:456–64.
28. Sinn N, Milte CM, Street SJ, *et al*. Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: a 6-month randomised controlled trial. *Br J Nutr* 2012;107:1682–93.
29. Stonehouse W. Does Consumption of LC Omega-3 PUFA Enhance Cognitive Performance in Healthy School-Aged Children and throughout Adulthood? Evidence from Clinical Trials. *Nutrients* 2014;6:2730–58.
30. Dangour AD, Allen E, Elbourne D, *et al*. Effect of 2-y n-3 long-chain polyunsaturated fatty acid supplementation on cognitive function in older people: a randomized, double-blind, controlled trial. *Am J Clin Nutr* 2010;91:1725–32.
31. Danthiir V, Hosking DE, Nettelbeck T, *et al*. An 18-mo randomized, double-blind, placebo-controlled trial of DHA-rich fish oil to prevent age-related cognitive decline in cognitively normal older adults. *Am J Clin Nutr* 2018;107:754–62.
32. Afzal S, Bojesen SE, Nordestgaard BG. Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. *Alzheimers Dement* 2014;10:296–302.
33. Annweiler C, Rolland Y, Schott AM, *et al*. Serum vitamin D deficiency as a predictor of incident non-Alzheimer dementias: a 7-year longitudinal study. *Dement Geriatr Cogn Disord* 2011;32:273–8.
34. Annweiler C, Schott AM, Rolland Y, *et al*. Dietary intake of vitamin D and cognition in older women: a large population-based study. *Neurology* 2010;75:1810–6.
35. Anastasiou CA, Yannakoulia M, Scarmeas N. Vitamin D and cognition: an update of the current evidence. *J Alzheimers Dis* 2014;42 Suppl 3:S71–S80.
36. Pettersen JA. Does high dose vitamin D supplementation enhance cognition?: A randomized trial in healthy adults. *Exp Gerontol* 2017;90:90–7.
37. Annweiler C, Beauchet O. Vitamin d in older adults: the need to specify standard values with respect to cognition. *Front Aging Neurosci* 2014;6.
38. Wu A, Ying Z, Gomez-Pinilla F. Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. *Neuroscience* 2008;155:751–9.
39. Andrieu S, Guyonnet S, Coley N, *et al*. Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. *Lancet Neurol* 2017;16:377–89.
40. Fielding RA, Vellas B, Evans WJ, *et al*. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc* 2011;12:249–56.
41. Tolea MI, Galvin JE. Sarcopenia and impairment in cognitive and physical performance. *Clin Interv Aging* 2015;10:663–71.
42. Boyle PA, Buchman AS, Wilson RS, *et al*. Association of muscle strength with the risk of Alzheimer disease and the rate of cognitive decline in community-dwelling older persons. *Arch Neurol* 2009;66:1339–44.
43. Kelaiditi E, Canevelli M, Andrieu S, *et al*. Frailty Index and Cognitive Decline in Alzheimer's Disease: Data from the Impact of Cholinergic Treatment USe Study. *J Am Geriatr Soc* 2016;64:1165–70.
44. Firth J, Stubbs B, Vancampfort D, *et al*. Grip Strength Is Associated With Cognitive Performance in Schizophrenia and the General Population: A UK Biobank Study of 476559 Participants. *Schizophr Bull* 2018;44:728–36.
45. Mavros Y, Gates N, Wilson GC, *et al*. Mediation of cognitive function improvements by strength gains after resistance training in older adults with mild cognitive impairment: Outcomes of the study of mental and resistance training. *J Am Geriatr Soc* 2017;65:550–9.
46. Nowson C, O'Connell S. Protein requirements and recommendations for older people: A review. *Nutrients* 2015;7:6874–99.
47. Daly RM, O'Connell SL, Mundell NL, *et al*. Protein-enriched diet, with the use of lean red meat, combined with progressive resistance training enhances lean tissue mass and muscle strength and reduces circulating IL-6 concentrations in elderly women: a cluster randomized controlled trial. *Am J Clin Nutr* 2014;99:899–910.
48. Morton RW, Murphy KT, McKellar SR, *et al*. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med* 2018;52:bjsp orts-2017-097608.
49. Mitchell CJ, Milan AM, Mitchell SM, *et al*. The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: a 10-wk randomized controlled trial. *Am J Clin Nutr* 2017;106:1375–83.
50. van de Rest O, van der Zwaluw NL, Tieland M, *et al*. Effect of resistance-type exercise training with or without protein supplementation on cognitive functioning in frail and pre-frail elderly: secondary analysis of a randomized, double-blind, placebo-controlled trial. *Mech Ageing Dev* 2014;136-137:85–93.
51. van de Rest O, van der Zwaluw NL, de Groot LCPGM. Literature review on the role of dietary protein and amino acids in cognitive functioning and cognitive decline. *Amino Acids* 2013;45:1035–45.
52. Chan AW, Tetzlaff JM, Altman DG, *et al*. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.
53. Schulz KF, Altman DG, Moher D. CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010;340:c332.
54. Boutron I, Moher D, Altman DG, *et al*. Extending the CONSORT statement to randomized trials of nonpharmacologic treatment: explanation and elaboration. *Ann Intern Med* 2008;148:295–309.
55. Borg G. Borg's Perceived Exertion and Pain Scales. Human Kinetics,1998 Champaign, IL, US
56. US Department of Health and Human Services. *The Report of the Dietary Guidelines Advisory Committee on Dietary Guidelines for Americans*. Washington, 2005.
57. Nowson CA, McGrath JJ, Ebeling PR, *et al*. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust* 2012;196:686–7.
58. Yang Y, Breen L, Burd NA, *et al*. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* 2012;108:1780–8.
59. Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull* 1988;24:709–11.
60. Nasreddine ZS, Phillips NA, Bédirian V, *et al*. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53:695–9.
61. Darby DG, Pietrzak RH, Fredrickson J, *et al*. Intraindividual cognitive decline using a brief computerized cognitive screening test. *Alzheimers Dement* 2012;8:95–104.
62. Hammers D, Spurgeon E, Ryan K, *et al*. Reliability of repeated cognitive assessment of dementia using a brief computerized battery. *Am J Alzheimers Dis Other Demen* 2011;26:326–33.
63. Maruff P, Thomas E, Cysique L, *et al*. Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. *Arch Clin Neuropsychol* 2009;24:165–78.
64. Bowie CR, Harvey PD. Administration and interpretation of the Trail Making Test. *Nat Protoc* 2006;1:2277–81.
65. Sánchez-Cubillo I, Periáñez JA, Adrover-Roig D, *et al*. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *J Int Neuropsychol Soc* 2009;15:438–50.
66. Giovagnoli AR, Del Pesce M, Mascheroni S, *et al*. Trail making test: normative values from 287 normal adult controls. *Ital J Neurol Sci* 1996;17:305–9.
67. Lovibond SH, Lovibond PF. *Manual for the Depression Anxiety Stress Scales*. Sydney, Australia: Psychology Foundation, 1995.
68. Ware JE, Gandek B. Overview of the SF-36 Health Survey and the International Quality of Life Assessment (IQOLA) Project. *J Clin Epidemiol* 1998;51:903–12.
69. Hawthorne G, Osborne RH, Taylor A, *et al*. The SF36 Version 2: critical analyses of population weights, scoring algorithms and population norms. *Qual Life Res* 2007;16:661–73.
70. Buysse DJ, Reynolds CF, Monk TH, *et al*. The pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193–213.
71. Daly RM, Miller EG, Dunstan DW, *et al*. The effects of progressive resistance training combined with a whey-protein drink and vitamin

D supplementation on glycaemic control, body composition and cardiometabolic risk factors in older adults with type 2 diabetes: study protocol for a randomized controlled trial. *Trials* 2014;15:431.
72. Dite W, Temple VA. A clinical test of stepping and change of direction to identify multiple falling older adults. *Arch Phys Med Rehabil* 2002;83:1566–71.
73. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc* 1991;39:142–8.
74. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res Q Exerc Sport* 1999;70:113–9.
75. World Health Organization. *Waist circumference and waist–hip ratio: Report of a WHO expert consultation*. Geneva, 2008. World Health Organization.
76. Nelson H E, Willison J. *National Adult Reading Test manual*. 2nd ed. Windsor, 1991. NFER-Nelson, Editor.
77. Bright P, Jaldow E, Kopelman MD. The National Adult Reading Test as a measure of premorbid intelligence: a comparison with estimates derived from demographic variables. *J Int Neuropsychol Soc* 2002;8:847–54.
78. Walsh SP, Raman R, Jones KB, *et al*. ADCS Prevention Instrument Project: the Mail-In Cognitive Function Screening Instrument (MCFSI). *Alzheimer Dis Assoc Disord* 2006;20(4 Suppl 3):S170–S178.
79. Amariglio RE, Donohue MC, Marshall GA, *et al*. Tracking early decline in cognitive function in older individuals at risk for Alzheimer disease dementia: the Alzheimer's Disease Cooperative Study Cognitive Function Instrument. *JAMA Neurol* 2015;72:446–54.
80. McCrae RR, Costa PTJ. *NEO Inventories: Professional manual*. Lutz, FL: Psychological Assessment Resources, Inc., 2010.
81. Slavin MJ, Brodaty H, Kochan NA, *et al*. Prevalence and predictors of “subjective cognitive complaints” in the Sydney Memory and Ageing Study. *Am J Geriatr Psychiatry* 2010;18:701–10.
82. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res* 1984;25:1391–6.
83. Smith JD. Apolipoproteins and aging: emerging mechanisms. *Ageing Res Rev* 2002;1:345–65.
84. Bennet AM, Di Angelantonio E, Ye Z, *et al*. Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA* 2007;298:1300–11.
85. Corder EH, Saunders AM, Strittmatter WJ, *et al*. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261:921–3.
86. Solomon A, Turunen H, Ngandu T, *et al*. Effect of the apolipoprotein e genotype on cognitive change during a multidomain lifestyle intervention: A subgroup analysis of a randomized clinical trial. *JAMA Neurol* 2018;75:462–70.
87. Stewart AL, Mills KM, King AC, *et al*. CHAMPS physical activity questionnaire for older adults: outcomes for interventions. *Med Sci Sports Exerc* 2001;33:1126–41.
88. Swierk M, Williams PG, Wilcox J, *et al*. Validation of an Australian electronic food frequency questionnaire to measure polyunsaturated fatty acid intake. *Nutrition* 2011;27:641–6.
89. GBD 2015 Neurological Disorders Collaborator Group. Global, *regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015*. *Lancet Neurol* 2017;16:877–97.
90. Ngandu T, Lehtisalo J, Solomon A, *et al*. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 2015;385:2255–63.
91. Montero-Odasso M, Almeida QJ, Burhan AM, *et al*. SYNERGIC TRIAL (SYNchronizing Exercises, Remedies in Gait and Cognition) a multi-Centre randomized controlled double blind trial to improve gait and cognition in mild cognitive impairment. *BMC Geriatr* 2018;18:93.
92. Lampit A, Valenzuela M. Pointing the FINGER at multimodal dementia prevention. *The Lancet* 2015;386:1625–6.