Australian
Atherosclerosis
Society Inc.

Annual Scientific Meeting

Programme and Abstracts

Monash CBD Conference Centre,
23rd - 25th October, 2024

Welcome

Welcome to Melbourne for what we believe will be a most memorable AAS Annual Scientific Meeting! It is, of course, the 50th anniversary of the establishment of the Australian Atherosclerosis Society.

We owe a huge debt of gratitude to Drs Leon Simons, Paul Nestel and Dennis Calvert, who co-founded the Australian Atherosclerosis Group in 1974, with the aim to form a non-profit association to promote the advancement of science, research and clinical management, in the field of atherosclerosis. The Australian Atherosclerosis Group remained very informal until the 1980's when it became incorporated with a constitution as the Australian Atherosclerosis Society (AAS). Dr Simons, Dr Nestel and Dr Calvert, working closely with other researchers in the lipid field, including Dr Philip Barter, Dr Noel Fidge and Dr Mark Wahlquist, convened two-day annual scientific meetings in Sydney, Melbourne, Canberra and Adelaide over the next 7-8 years. From its inception, the AAS has been internationally recognised and has hosted two International Symposia on Atherosclerosis. AAS members have significant international profiles in both the clinical and scientific spheres.

This year, the AAS Scientific Program includes a fabulous line-up of invited International and National speakers including: Prof Muredach Reilly from Columbia University NY, USA; Prof Marianne Benn from the Uni Copenhagen, Denmark (via Zoom); Prof Jong-Chan Youn, Catholic Uni of Korea, South Korea; Prof Kerry-Anne Rye, Uni NSW; and Prof Peter Psaltis, SAHMRI and Uni Adelaide.

Once again, we will have both invited and free communication sessions – all oral – ensuring that there will be something for everyone.

All AAS sessions will be held at the Monash Conference Centre, in the Melbourne CBD.

Meetings like this, including all the activities that we have hosted over the past year, would not be possible without the hard work and dedication of the members of the AAS Executive Committee, who make my job look easy! Indeed, once again, this face-to-face meeting has been organised wholly by the committee, embracing our "by members, for members" approach. Therefore, I would like to extend my deepest gratitude to the Program Organising Committee who have tirelessly devoted time to organise this meeting.

We are equally grateful for the ongoing support of our education program partners, AMGEN, Novartis, and CSL, whose continued sponsorship reflects the value they place on advancing scientific research in cardiovascular medicine. Their contributions have played a vital role in facilitating the exchange of ideas and fostering new collaborations for the treatment of CVDs.

This year's celebratory meeting holds special significance as we bring together past presidents for a unique discussion to highlight how far we have come in the development of innovative treatments for cardiovascular health, serving as a testament to the progress we have made over the past five decades. Be sure not to miss this unique panel discussion on the final day of the conference!

We are sure that this very special meeting in Melbourne will prove to be a memorable occasion in the cardiovascular calendar and we hope that you will benefit from the marvellous opportunities for catching up with good friends and sharing great science.

As we approach 2025, the AAS is excited for the possibilities that lie ahead. It remains our mission to strengthen scientific collaborations, foster young and emerging talent, and continue to educate via our Clinical masterclasses. Additionally, we look forward to welcoming new members onto our Executive board, as we bid others farewell who have contributed greatly. Undoubtedly, fresh perspectives and new ideas will strengthen the focus of our committee so that we can continue to bring you the most innovative and up-to-date CV research content, both from within Australia and globally, via our educational events.

Wishing you all an inspiring and educational 2024 ASM!

AAS President, Professor Judy de Haan

Atherosclerosis Education Partners

The Australian Atherosclerosis Society wishes to thank and acknowledge our 2024 Atherosclerosis Education Program Partners, Amgen and Novartis, for supporting all our educational activities including the Scientific Showcase Seminars, Annual Scientific Meeting, Clinical Masterclass and FH Summit.

We also acknowledge CSL, the supporter of our Rising Star Awards for the Annual Scientific Meeting.

AMGEN NOVARTIS CSL™ RISING STAR AWARD

2024 Committees

AAS Executive Committee	Program Committee	Abstract Reviewers
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Stephen Nicholls (Past-President)	Joseph Moxon (Co-Convenor)	Costan Magnussen (Baker)
Kristen Bubb (Secretary)	Joanne Tan (Past-Convenor)	Dragana Dragoljevic (Baker)
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Joanne Tan (Sponsorship)	Emma Solly (SCOLAR)	Steven Gieseg (Canterbury)
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Costan Magnussen		Emma Solly (SAHMRI)
Carl Schultz		Peter Psaltis (SAHMRI)
Carl Schultz		Maaike Kockx (Sydney)
		Brad Tucker (Sydney)
		Mark Nelson (Tasmania)
		Natalie Ward (UWA)
		Heather Medbury (Westmead)

Program at a glance

Wednesday 23rd October	
0900 – 1000	Registration opens
1000 – 1130	SCOLAR PROGRAM
1130 – 1245	ECR Lunch with Experts (registration required)
1300 – 1315	President's Welcome
1315 – 1440	Session 1: National Plenary & Lipoproteins in Cardiometabolic Disease
1440 – 1500	Afternoon tea
1500 – 1605	Session 2: Mechanisms of Disease – Endothelial Cells & Efferocytosis
1605 – 1730	Welcome Drinks

Thursday 24th October	
0900 – 1030	Session 3: International Plenary & Mechanisms of Disease
1030 – 1045	Morning tea
1045 – 1215	Session 4: HDR Rising Star Finalists
1215 – 1315	Lunch
1315 – 1500	Session 5: Novel Approaches in Atherosclerosis
1500 – 1515	Afternoon tea
1515 – 1650	Session 6: International Plenaries & Clinical Cardiometabolic Complications
1700 – 1745	Annual General Meeting
1900 – 2100	ASM Dinner – 50th Anniversary Celebrations

Friday 25th October	
0900 – 1030	Session 7: National Plenary & ECR Rising Star Awards
1030 – 1045	Morning tea
1045 – 1215	AAS Presidents' Panel Atherosclerosis Research in Australia: Past, Present and Future
1215 – 1230	Presentation of Rising Star Awards and Meeting Close

Program details

Wednesday 23rd October			
SCOLAR PROGRAM			
Chairs: Dr Arpeeta Sharma & Dr Emma Solly			
1000	**S1**	Genomics in translation of clinical heart disease	Prof Muredach P. Reilly
1020	**S2**	Diabetic cardiovascular complications	Prof Rebecca Ritchie
1040	**S3**	Targeting atherosclerosis with RNA therapy	Prof Merlin Thomas
1100 – 1130		Panel Discussion	
1130 – 1245		Lunch with Experts (registration required)	

Session 1: National Plenary & Lipoproteins in Cardiometabolic Disease			
Chairs: Prof Andrew Murphy & Dr Siân Cartland			
1300		President's Welcome	Prof Judy de Haan
1315	**1**	**National Plenary #1:** Current and future therapeutic options for reducing cardiometabolic disease	Prof Kerry-Anne Rye
1345	**2**	HDL functionality as a biomarker for risk of coronary artery disease and diabetes in Indigenous Australians	Dr Joanne Tan
1400	**3**	HDL remodelling and enrichment with apoCIII and apoE in Indigenous Australians	Dr Maaike Kockx
1415	**4**	Cardiovascular events in patients with diabetes-related foot ulcers: does inflammation and infection drive adverse outcomes?	Dr Nick Si Rui Lan
1430	**5**	Enhancing anti-inflammatory stimuli to prevent atherosclerosis progression through macrophage-specific RIPK1	Mr Han Xu
1435	**6**	Current understanding of lipoprotein (a) and its impact on peripheral vascular disease: A narrative literature review	Dr Amirul Hakim Ahmad Bazlee
1440	Afternoon tea		

Session 2: Mechanisms of Disease - Endothelial Cells & Efferocytosis			
Chairs: Dr Kristen Bubb & A/Prof Joseph Moxon			
1500	**7**	Females exhibit greater mitochondrial endothelial dysfunction, impairing vascular healing in diabetes-associated peripheral artery disease	A/Prof Mary Kavurma
1515	**8**	The generation of stable microvessels in ischaemia is mediated by endothelial cell derived TRAIL	Dr Siân Cartland
1530	**9**	Death out of balance: a computational study of cell death and defective efferocytosis in atherosclerotic plaque tissue	Dr Ishraq Ahmed
1545	**10**	Examining the role of vascular endothelial cells in efferocytosis	Dr Amy Baxter
1600	**11**	Hypoxia-responsive nanoparticle mediated Delivery of the antimiR-181c to reverse diabetes-impaired angiogenesis	Dr Joanne Tan
1605	Welcome Drinks		

Thursday 24th October			
Session 3: International Plenary & Mechanisms of Disease			
Chairs: Dr Denuja Karunakaran & Dr. Heather Medbury			
0900	**12**	**International Plenary #1 (AAS Sponsored):** Genomics drives novel concepts in atherosclerotic cardiovascular diseases	Prof Muredach P. Reilly
0945	**13**	Genetic and biochemical association of the SLC22A3 transporter with Lp(a) levels and uptake in liver cells	Prof Sally McCormick
1000	**14**	The inflammatory profile of human monocyte-derived macrophages is associated with blood lipid levels	Dr Heather Medbury
1015	**15**	Gene expression profiling of myocardial ischaemic preconditioning and myocardial stunning	Dr Waheed Khan
1030	Morning Tea		

Session 4: HDR Rising Star Awards			
Chairs: A/Prof Steven Gieseg & Dr Blake Cochran			
1045	**16**	Selectively targeting the Gasdermin-D pore attenuates cardiac inflammation and fibrosis after ischemia reperfusion injury	Ms Judy Choi
1100	**17**	Understanding the cellular and transcriptomic landscape of diabetes-associated peripheral artery disease by single-cell RNA sequencing	Ms Elaina Kelland
1115	**18**	Modulation of endothelial-to-mesenchymal transition by an epigenetic drug GSK-126 attenuates diabetes associated atherosclerosis	Ms Misbah Aziz
1130	**19**	Therapeutic *Akkermansis muciniphila* supplementation to enhance atherosclerotic plaques stability in the tandem stenosis mouse model	Ms Marziyeh Anari
1145	**20**	Atheroprotective effects of GLP-1 delivered by DNA minicircle	Mr Gardner Robinson
1200	**21**	A novel immunometabolic therapy for diabetic cardiovascular complications	Mrs Parvin Yavari
1215	Lunch		

Session 5: Novel Approaches in Atherosclerosis			
Chairs: Prof Sally McCormick & Dr Amy Baxter			
1315	**22**	Mechanotransduction in immune cells: The hidden driver in aortic stenosis	A/Prof Sara Baratchi
1330	**23**	Resolving inflammation by targeting FPR2 protects against diabetes associated atherosclerosis	Ms Yvonne Zhang
1345	**24**	Lipoxin mediates resolution of diabetes-associated atherosclerosis (DAA) in ApoE-/- diabetic mice via a direct effect on perivascular adipose tissue (PVAT)	Dr Phillip Kantharidis
1400	**25**	Development of a 3D-Printed tandem stenosis model for consistent induction of plaque instability in *ApoE*$^{-/-}$ mice	Dr Yung-Chih (Ben) Chen
1415	**26**	Trimethylamine-N-Oxide's role in atherosclerotic plaque stability: Insights from fluoromethylcholine studies in a tandem stenosis mouse model	Ms Yi Hua

1430	**27**	In situ measurement of live human plaque permeability and cell infiltration using spectral photon counting CT imaging	A/Prof Steven Gieseg
1445	**28**	The utility of Computed Tomography Coronary Angiography for predicting major adverse cardiac events in patients undergoing investigation for chest pain: a single-centre study	Mr Thomas Faulder
1450	**29**	A new mathematical model for plaque growth	Prof Mary Myerscough
1455	**30**	A mathematical model for the role of smooth muscle cells phenotype switching in atherosclerotic plaques	Dr Joseph Ndenda
1500	Afternoon Tea		

Session 6: International Plenaries & Clinical Cardiometabolic Complications			
Chairs: Prof Judy de Haan & Dr Stjepana Maticevic			
1515	**31**	**International Plenary #2 (AAS & KSoLA Partnership):** Pathogenic role of senescent T cells in cardiovascular disease	Prof Jong-Chan Youn
1540	**32**	Triglycerides contribute to the pro-inflammatory actions of neutrophil extracellular traps	Dr Blake Cochran
1555	Mini break – Zoom setup		
1600	**33**	**International Plenary #3 (AAS & EAS Partnership):** Obesity and cardiovascular disease risk	Prof Marianne Benn
1630	**34**	Elevated lipoprotein(a) and familial hypercholesterolaemia in the coronary care unit at Sir Charles Gairdner Hospital	Dr Julian Atlas
1645	**35**	Low-density lipoprotein cholesterol estimation in youth: Sampson equation superior in predicting mid-adult carotid plaque	Dr Yaxing Meng
1650	Mini break		
1700	Annual General Meeting		
1900	ASM Dinner – 50th Anniversary Celebrations		

celebrating 50 YEARS

Australian Atherosclerosis Society Inc.

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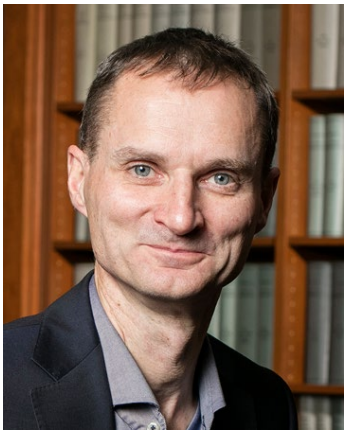
Friday 25th October			
Session 7: National Plenary & ECR Rising Star Awards			
Chairs: Dr Nick Lan & Dr Yung-Chih (Ben) Chen			
0900	**36**	**National Plenary #2:** From fundamental discovery to clinical practice: targeting old and new players in vascular disease	A/Prof Peter Psaltis
0930	**37**	The functionality of high-density lipoproteins is impaired in patients with diabetes that have undergone toe amputations	Dr Emma Solly
0945	**38**	Diabetes induces TET2 dysfunction in bone marrow haematopoietic stem cells and accelerates DNMT3A R878H/+ clonal haematopoiesis in mice	Dr Dragana Dragoljevic
1000	**39**	Differential gene expression patterns during apoptotic and necroptotic cell clearance by primary macrophages	Dr Narmadaa Thyagarajan
1015	**40**	PCSK9 AAV delivery to induce hypercholesterolemia in the tandem stenosis mouse model of plaque instability	Dr Jordyn Thomas
1030	Morning Tea		

AAS Presidents' Panel Atherosclerosis Research in Australia: Past, Present and Future	
Chair: Prof Judy de Haan	
1045 – 1215	This final panel brings together founding member Prof Paul Nestel & 6 AAS past presidents: Prof Kerry-Anne Rye, Prof Richard O'Brien, Prof Gerald Watts, Prof David Sullivan, A/Prof Christina Bursill & A/Prof Peter Psaltis who will share their thoughts on the past, present and future of atherosclerosis research in Australia.
1215 – 1230	Presentation of Rising Star Awards and Meeting Close

Invited International Plenary Speakers



Prof. Muredach P. Reilly, Columbia University, USA

Genomics drives novel concepts in atherosclerotic cardiovascular diseases

Dr. Muredach P. Reilly received his medical degree from University College Dublin, Ireland and completed his residency and fellowship training in Medicine and Cardiovascular Medicine at the University of Pennsylvania, where he received a M.S. degree in clinical & genetic epidemiology.

Dr. Reilly is the Herbert and Florence Irving Professor of Medicine and Vice Dean for Clinical and Translational Research at Columbia University Irving Medical Center in New York, USA. He is Director of the Irving Institute for Clinical and Translational Research, and PI of Columbia University's NIH funded Clinical and Translational Science Award (CTSA) Program hub. He is also a Vice Dean for Research at the Vagelos College of Physicians and Surgeon at Columbia University, New York, USA.

Dr. Reilly serves as the Director of the Cardiometabolic Precision Medicine Program in the Division of Cardiology at Columbia University. His research program is dedicated to translational precision medicine studies of human atherosclerosis and heart disease as well as inflammatory mechanisms in cardio-metabolic disease, emphasizing humans as the most ideal "model" to understand mechanisms of and therapeutic opportunities for human disease and prevention.

Prof. Marianne Benn, University of Copenhagen, Denmark

Obesity and cardiovascular disease risk

Professor Marianne Benn, MD, PhD and DMSc is senior consultant at Department of Clinical Biochemistry, Rigshospitalet, Copenhagen; Professor in Clinical Biochemistry with special focus on Translational Medicine; and co-chair of the BRIDGE – Translational Excellence Programme at University of Copenhagen, Denmark.

Her main research interest is genetic epidemiology using Mendelian randomization designs to examine the unconfounded effects of environmental factors and biological markers on risk of cardiovascular disease in the general population, for prediction of unintended effects of drugs, and examining the contributions and mechanisms of risk factors such as obesity, LDL cholesterol, diabetes to micro- and macrovascular disease. Prof Benn will join us online via Zoom (in partnership with the European Atherosclerosis Society).

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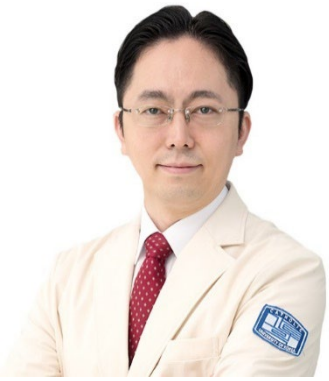


Prof. Jong-Chan Youn, Catholic University of Korea

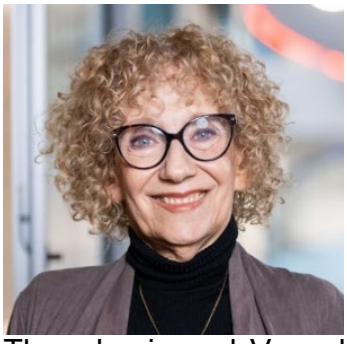
Pathogenic role of senescent T cells in cardiovascular disease

Dr. Jong-Chan Youn is a Professor at the Division of Cardiology, Seoul St. Mary's Hospital, The Catholic University of Korea. After graduating from Yonsei University College of Medicine, he obtained his Ph.D. from the same institution. He then worked as a full-time postdoctoral researcher at KAIST's Immunology Lab, where he focused on cardiovascular immunology and senescent T cell research.

Dr. Youn has published a series of articles on senescent T cells and their impact on human hypertension, arterial stiffening, myocardial infarction, and heart failure. His clinical expertise encompasses hypertension, dyslipidaemia, and heart failure. Dr Youn's conference attendance is partially supported by Korean Society of Lipids and Atherosclerosis (KSoLA).

KSoLA
The Korean Society of Lipid and Atherosclerosis

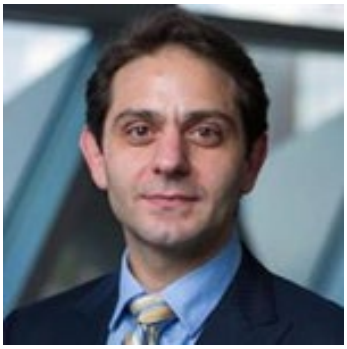
Invited National Plenary Speakers



Prof. Kerry-Anne Rye, University of New South Wales

Current and future therapeutic options for reducing cardiometabolic disease

Professor Kerry-Anne Rye (BSc (Hons), PhD, FAHA) is Deputy Head of the School of Biomedical Sciences and Head of the Cardiometabolic Research Group in the Faculty of Medicine at UNSW Sydney. She is the Immediate Past-Chair of the American Heart Association Arteriosclerosis, Thrombosis and Vascular Biology (ATVB) Council Nominations Committee, Editor-in-Chief of the Journal of Lipid Research and Senior Editor of the Journal of the American Heart Association. Professor Rye is recognised internationally for her work on high density lipoprotein (HDL) structure and function and cardiometabolic disease. She has published over 300 peer-reviewed papers on these topics in discipline-specific and general journals. Her current research focus is on the development of novel, therapies for treatment of diabetes-accelerated atherosclerosis.



A/Prof. Peter Psaltis, SAHMRI & University of Adelaide

From fundamental discovery to clinical practice: targeting old and new players in vascular disease

A/Prof Peter Psaltis is an NHF- and NHMRC-funded interventional cardiologist and vascular biologist, who holds Faculty positions at SAHMRI, CALHN and the University of Adelaide. He is the Deputy Director of SAHMRI, co-leads its largest research department, the Lifelong Health Theme of more >310 researchers, and leads its Heart and Vascular Program. Concurrently, he is also Head of Interventional Cardiology, Royal Adelaide Hospital. Peter has expertise across all three disciplines of basic, translational, and clinical research and leads bench-to-bedside projects spanning topics of developmental macrophage biology, vascular stem cells, inflammatory regulation of atherosclerosis, pharmacological modification of atherosclerosis, coronary plaque imaging, the modelling of biomechanical forces in coronary arteries and cardiometabolic disease.

2

HDL functionality as a biomarker for risk of coronary artery disease and diabetes in Indigenous Australians

Joanne Tan[1,2], Esma Vorrasi[1,2], Victoria Nankivell[1,2], Emma Solly[1,2], Thalia Salagaras[1], Natasha Howard[2,3], Alex Brown[4,5], Christina Bursill[1,2], Peter Psaltis[1,2]

[1]Vascular Research Centre, Lifelong Health Theme, South Australian Health and Medical Research Institute, Adelaide, Australia. [2]Adelaide Medical School, University of Adelaide, Adelaide, Australia. [3]Implementation Science Program, Wardliparingga Aboriginal Health Equity Theme, South Australian Health and Medical Research Institute, Adelaide, Australia. [4]Telethon Kids Institute, Adelaide, Australia. [5]Australian National University, Canberra, Australia

Background: Indigenous Australians experience higher rates of coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) compared to non-Indigenous Australians. High-density lipoproteins (HDL) exert cardioprotective properties, including cholesterol efflux and anti-inflammatory effects. Evidence suggests that HDL function is impaired in CAD and T2DM.

Aim: To determine if HDL functionality is reduced in Indigenous Australians.

Methods & Results: HDL was isolated from Non-Indigenous Healthy, Indigenous Healthy, Indigenous+CAD and Indigenous+CAD+T2DM serum (n=10/group). Cholesterol efflux capacity (CEC) in [^{3}H]-cholesterol-loaded macrophages was increased with HDL from Non-Indigenous Healthy (3.9±0.6%), Indigenous+CAD (3.5±0.5%) and Indigenous+CAD+T2DM (3.6±0.6%) groups compared to baseline (2.6±0.1%, P<0.05 for all). However, this increase was not seen with Indigenous Healthy HDL, which had reduced CEC compared to Non-Indigenous Healthy (3.2±0.5%, P<0.05). Furthermore, in human coronary artery endothelial cells, Non-Indigenous Healthy HDL attenuated TNFα-stimulated expression of inflammatory transcription factor p65 NFκB (*RELA*) and chemokines *CCL5* and *CX3CL1* (P<0.05 for all). Importantly, this anti-inflammatory effect was blunted for healthy Indigenous HDL. Interestingly, Indigenous+CAD+T2DM HDL suppressed TNFα-induced *CX3CL1* and cell adhesion molecules *VCAM1* and *ICAM1* (P<0.05 for all). Finally, using a Boyden chamber assay, monocyte migration was reduced in HDL-treated conditioned media across all 4 groups compared to TNFα-stimulated conditioned media alone (P<0.0001 for all).

Conclusions: HDL functionality was reduced in Indigenous Healthy group compared to Non-Indigenous Healthy group. However, HDL functionality was improved in Indigenous+CAD and Indigenous+CAD+T2DM groups. Impaired HDL functionality may predispose Indigenous Peoples to the detrimental effects of inflammation, driving CAD and T2DM progression.

3

HDL remodelling and enrichment with apoCIII and apoE in Indigenous Australians

Maaike Kockx[1,2], Jeffrey Wang[1,2], Natasha Howard[3,4], Avinash Suryawanshi[5], Stephen Nicholls[6], Alex Brown[3,7], Leonard Kritharides[8,2,9]

[1]ANZAC Research Institute/Concord Hospital, Sydney, Australia. [2]University of Sydney, Sydney, Australia. [3]South Australian Health and Medical Research Institute, Adelaide, Australia. [4]University of Adelaide, Adelaide, Australia. [5]Concord Hospital, Sydney, Australia. [6]Victorian Heart Institute/Monash University, Melbourne, Australia. [7]National Centre for Indigenous Genomics/John Curtin School of Medicial Research/Australian National University, Canberra, Australia. [8]ANZAC Research Institute, Sydney, Australia. [9]Department of Cardiology/Concord Hospital, Sydney, Australia

Indigenous Australians have an increased risk of type 2 diabetes mellitus (T2DM) and premature cardiovascular disease. Subpopulations of high density lipoprotein (HDL) cholesterol have been associated with increased cardiovascular risk, but HDL composition, size or function have not been studied in Indigenous Australians.

The study consisted of 161 subjects (86 non-Indigenous 43 of whom had T2DM, and 75 Indigenous 36 of whom had T2DM) without known coronary disease. HDL lipid and apolipoprotein content were determined using enzymatic assays and ELISA respectively, and HDL size and distribution were investigated using nuclear magnetic resonance spectroscopy. Transporter-independent, ATP-binding cassette transporter-A1 (ABCA1)- and -G1 (ABCG1)-specific cholesterol efflux capacity (CEC), validated measures of HDL function, were determined using cell lines stably expressing human ABCA1 or ABCG1.

Indigenous Australians were younger, had higher rates of smoking, higher BMI, higher plasma triglycerides (TG), lower HDL-C and lower apolipoprotein A-I (apoA-I) than non-Indigenous Australians. Indigenous Australians had lower total HDL particle numbers (17.6±3.4 v 20.5±2.7 μmol/l, $p<0.001$), decreased large and small HDL particles, with large 10.3nm particle concentrations 71% lower in Indigenous subjects ($p<0.001$). HDL particles from Indigenous Australians were highly enriched in TG, apoE and apoCIII (all $p<0.001$), and HDL-TG levels were higher in those with concurrent T2DM. After adjusting for apoA-I levels, transporter-independent and ABCG1-mediated CEC were not different, whereas ABCA1-specific CEC was higher in Indigenous than in non-Indigenous subjects (0.83±0.02 v 0.94±0.01, $p<0.001$). Multivariable analysis identified that ABCA1-specific CEC was independently and positively associated with HDL-apoCIII and HDL-apoE levels.

4

Cardiovascular events in patients with diabetes-related foot ulcers: does inflammation and infection drive adverse outcomes?

Nick S. R. Lan[1,2,3], Jonathan Hiew[1], Ivana Ferreira[1], Carsten Ritter[1,4], Laurens Manning[1,2], Gerry Fegan[1,4], Girish Dwivedi[1,2,3], Emma J. Hamilton[1,2]

[1]Fiona Stanley Hospital, Perth, Australia. [2]The University of Western Australia, Perth, Australia. [3]Harry Perkins Institute of Medical Research, Perth, Australia. [4]Curtin University, Perth, Australia

Objective: Diabetes-related foot ulceration (DFU) is associated with increased cardiovascular risk, but the mechanisms remain unclear. Inflammation and infection potentiate cardiovascular disease which may be important in DFU.

Methods: Prospectively collected data from a multidisciplinary DFU service were analysed. A deep ulcer was defined as reaching muscle, tendon or deeper. Patients were categorised into four DFU groups: not deep and no infection (D-I-), not deep but infected (D-I+), deep with no infection (D+I-), or deep with infection (D+I+). Incident major adverse cardiovascular events (MACE) was defined as hospitalisation for myocardial infarction, stroke or transient ischaemic attack, or heart failure. Survival analyses and multivariate cox regression were used.

Results: Of 513 patients, 241 (47.0%) were D-I-, 110 (21.4%) D-I+, 35 (6.8%) D+I- and 127 (24.8%) D+I+. MACE or all-cause mortality occurred in 75 (14.6%) patients and MACE only in 46 (9.0%) after median follow-up of 381 (interquartile range [IQR] 220-551) and 404 (IQR 228-576) days, respectively. Infection was associated with significantly higher MACE or all-cause mortality (21.5%vs8.7%; p<0.001) and MACE (13.5%vs5.1%; p=0.003). MACE or all-cause mortality was significantly higher in D+I+ (D-I- 7.9%; D-I+ 15.5%; D+I- 14.3%; D+I+ 26.8%; p<0.001), as was MACE (D-I- 5.0%; D-I+ 10.9%; D+I- 5.7%; D+I+ 15.7%; p=0.017). Infection and deep ulcer were independent predictors of adverse outcomes.

Conclusions: Deep and/or infected DFUs are associated with increased cardiovascular risk compared with DFU that is not deep or infected. These findings provide a potential mechanistic explanation requiring investigation.

5

Enhancing anti-inflammatory stimuli to prevent atherosclerosis progression through macrophage-specific RIPK1

Han Xu[1,2], Massaki Sato[1,2], Narmadaa Thyagarajan[1,2], Alex Pokrassen[1,2], Taaseen Rahman[1,2], Vik Ven Eng[1,2], Yizhuo Wang[3], Kavita Bisht[3], Blake Cochran[4], Kerry-Anne Rye[4], Denuja Karunakaran[1,3,2]

[1]Department of Physiology, Monash University, Clayton, Australia. [2]Victorian Heart Hospital, Clayton, Australia. [3]Institute of Molecular Biology, The University of Queensland, Queensland, Australia. [4]University of New South Wales, Sydney, Australia

Background: The CANTOS trial has instigated targeting inflammation to treat atherosclerosis to the forefront of new therapies. We have shown Receptor interacting protein kinase-1 (RIPK1) as a key regulator of oxidised LDL (oxLDL)-mediated NF-κB-dependent inflammation during early atherosclerosis. In contrast, anti-inflammatory agents (e.g. cytokine IL-10 or IL-13) and high-density lipoprotein (HDL), and its key protein component, apolipoprotein-AI (ApoAI), reduce inflammation within lesions. Herein, we aim to investigate whether these anti-inflammatory agents target macrophage RIPK1 functions and the resultant atherosclerosis.

Methods: Bone marrow-derived macrophages (BMDMs) were isolated from *Ripk1*$^{+/-}$ or wild-type (Wt) littermate mice for *in vitro* studies. *In vivo*, *Ldlr*$^{-/-}$ mice (male & female, n=8-9/group) were transplanted with either *Ripk1*$^{+/-}$ or Wt bone marrow and were fed a western diet for 16 weeks.

Results: *Ripk1*$^{+/-}$ BMDMs have reduced mitochondrial superoxide production (MitoSox) in response to oxLDL or aggregated LDL ($p<0.01$), compared to Wt, indicating Ripk1 promote inflammation via mitochondrial oxidative stress. Interestingly, in Wt BMDMs, both 100ng/mL Il-10 or Il-13, reduce Ripk1 gene and protein expression ($p<0.01$). Inhibition of transcription factor, STAT3, reverses Il-10-induced Ripk1 reduction, implicating a vital role of IL-10-STAT3 axis in negatively regulating Ripk1. Further, HDL and ApoAI, independent of its known protein kinase A-signaling, also reduce *Ripk1* and *Nf-kb* (p65) expression ($p<0.001$). In vivo, bone marrow transplant of *Ripk1*$^{+/-}$ into *Ldlr*$^{-/-}$ mice reduced aortic sinus lesion area after 16-week western diet feeding relative to Wt controls ($p<0.05$).

Conclusions: Together, this data highlights novel physiological mechanisms that repress macrophage Ripk1, presenting an alternative strategy to treat atherosclerosis.

6

Current understanding of lipoprotein (a) and its impact on peripheral vascular disease: A narrative literature review

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Lipoprotein(a) [Lp(a)], a complex type of lipoprotein and shares structural similarities with low-density lipoprotein (LDL aside from the unique presence of apolipoprotein(a) [apo(a)] linked to apolipoprotein B-100. While the physiological role of Lp(a) is not yet fully understood, evidence suggests its potential to expedite wound healing, facilitate tissue repair, and impede cancer growth and metastasis. Lipoprotein Lp(a) also demonstrates proatherogenic traits in its capacity to convey pro-inflammatory oxidized phospholipids (OxPL) and foster the secretion and expression of pro-inflammatory cytokines, augments endothelial cell permeability, stimulates smooth muscle cell migration and proliferation, and induces the expression of adhesion molecules, leading to monocyte recruitment and retention. Furthermore, Lp(a) demonstrates thrombogenic activities by promoting platelet aggregation, impeding plasminogen activation, and exerting inhibitory effects on fibrinolysis.

Numerous studies have underscored the significance of elevated Lp(a) levels as an independent causal risk factor for cardiovascular disease (CVD), though its significance in PAD has not yet fully elucidated. This narrative review intends to meticulously present and thoroughly examine the epidemiologic and clinical evidence related to the effects of Lp(a) on PAD, considering a wide range of studies and findings in the field. Understanding the complex mechanisms by which Lp(a) contribute to PAD and its development is essential for developing precise and effective treatments tailored to the needs of individual patients.

7

Females exhibit greater mitochondrial endothelial dysfunction, impairing vascular healing in diabetes-associated peripheral artery disease

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Peripheral Artery Disease (PAD) is the leading cause of limb pain, non-healing ulcers and amputation, increasing the risk of heart attack and stroke. Diabetes is a major risk factor. Women with diabetes-associated PAD experience greater graft failure and limb loss, functional impairment, reduced long-term survival after revascularization and increased post-surgical mortality when compared to men. Why this is the case is unclear. We aimed to identify whether sexual dimorphisms in endothelial cell (EC) function(s) impact diabetes-associated PAD pathophysiology. Male and female *Apoe*$^{-/-}$ mice were rendered diabetic with streptozotocin followed by hindlimb ischaemia (HLI) 18w later. Mice were euthanised 2w post-HLI. While plasma glucose levels and glucose tolerance were equivalent between sexes, diabetic female tissues had impaired angiogenesis and arterial relaxation and greater inflammation and oxidative stress in response to HLI. Single cell RNA sequencing of gastrocnemius revealed 3 EC clusters and genes regulating mitochondrial function were significantly reduced in female ECs from diabetes-associated PAD mice. Remarkably, amputation tissues from female diabetes-associated PAD patients showed ~50% reduction in stable microvessel numbers and impaired arterial relaxation compared to males. Female patient tissues also had increased inflammation, oxidative stress and reduced expression of mitochondrial genes. Our findings suggest that mitochondrial EC dysfunction could be the driving force for poorer vascular health in female patients and could explain why women with diabetes-associated PAD have worse outcomes to treatment. Improving mitochondrial EC function(s) could represent a target for sex-specific therapy.

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The generation of stable microvessels in ischaemia is mediated by endothelial cell derived TRAIL

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Reversal of ischaemia is mediated by neo-angiogenesis requiring endothelial cell (EC) and pericyte interactions to form stable microvascular networks. Here we describe an unrecognised role for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in potentiating neo-angiogenesis and vessel stabilization. We show the endothelium is a major source of TRAIL in the healthy circulation compromised in peripheral artery disease (PAD). EC-deletion of TRAIL *in vivo* or *in vitro* inhibited neo-angiogenesis, pericyte recruitment, and vessel stabilization, resulting in reduced lower-limb blood perfusion with ischaemia. Activation of the TRAIL receptor (TRAIL-R) restored blood perfusion and stable blood vessel networks in mice. Proof-of-concept studies showed that Conatumumab, an agonistic TRAIL-R2 antibody, promoted vascular sprouts from explanted patient arteries. Single cell RNA-sequencing revealed heparin binding-EGF-like growth factor in mediating EC-pericyte communications dependent on TRAIL. These studies highlight unique TRAIL-dependent mechanisms mediating neo-angiogenesis and vessel stabilization, and the potential of repurposing TRAIL-R2 agonists to stimulate stable and functional microvessel networks to treat ischaemia in PAD.

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Death out of balance: a computational study of cell death and defective efferocytosis in atherosclerotic plaque tissue

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In atherosclerosis and other inflammatory diseases, the resolution of inflammation involves a dynamic balance between cell death and the clearance of dying cells via efferocytosis. In nonresolving plaques, this balance is disrupted due to the accumulation of toxic amounts of free cholesterol by macrophages, which impairs their efferocytic ability and causes higher rates of both apoptotic and necrotic cell death.

Here we present a cell-based simulation model of macrophages in plaque tissue. The model incorporates cell death via apotosis, primary necrosis, and post-apoptotic necrosis, efferocytic clearance of dead cells, and cell movement. We analyse the model's long-term behaviour, and we find that low rates of cell death and high rates of efferocytic uptake are required to allow live cells and dead cells to coexist. We find in particular that high rates of necrosis and poor efferocytic uptake of necrotic material can cause tissue necrosis even in cases where efferocytosis can keep up with apoptotic death.

We also present a time-averaged model for the relative proportions of live, apoptotic, and necrotic cells. This model allows for deeper quantitative insights into how the relative rates of death and efferocytosis affect plaque composition. We find that for certain parameter values, the long-term tissue composition can settle to either a necrotic or a benign coexistent state depending on the initial availability of live cells. We also find time-sensitive behaviour where the gradual impairment of efferocytic ability can cause permanent necrosis of initially healthy tissue, even after efferocytic ability has been restored.

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Examining the role of vascular endothelial cells in efferocytosis

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The removal of dying cells by phagocytes known as ‘efferocytosis’ is a critical process to maintain tissue homeostasis. Although endothelial cells (ECs) can engulf pathogens, lipoproteins and erythrocytes by phagocytosis, their participation in efferocytosis of dying ECs (e.g. neighbouring cells within the vessel wall) is only beginning to be defined. A better understanding of the mechanisms and downstream consequences of EC efferocytosis could lead to novel therapeutic strategies to promote vessel repair in diseases associated with elevated EC apoptosis, such as atherosclerosis. Examining the efferocytic properties of human and mouse ECs by flow-cytometry and confocal microscopy revealed that ECs are efficient phagocytes *in vitro*. The ability of ECs to perform efferocytosis under physiological sheer stress and pressure conditions was also confirmed, using a bio-fluidics approach. To visualise EC efferocytosis in a living system, a zebrafish model was employed. Apoptotic cell fragments injected intravascularly into embryos trafficked to regions of high vascularisation where they were engulfed by ECs, demonstrating EC efferocytosis *in vivo*. The impact of efferocytosis by ECs on tissue homeostasis was determined by RNAseq. Over time, upregulation of gene signatures associated with glucose metabolism, nutrient transport, growth and repair were identified in engulfing ECs, correlating with enhanced metabolic activity and enhanced efferocytosis capacity. This data suggests that ECs may function to replenish damaged tissue following sensing and internalisation of apoptotic material. Together, these findings highlight an emerging role of ECs as mediators of apoptotic EC clearance that may lead to novel therapeutic targets of vessel damage.

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Hypoxia-responsive nanoparticle mediated delivery of the antimiR-181c to reverse diabetes-impaired angiogenesis

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Background: Diabetic vascular complications are characterised by impaired angiogenic responses to ischaemia and wound healing. MicroRNAs simultaneously regulate a host of cellular signalling pathways making them ideal targets for correcting multifaceted diseases. We recently found that inhibition of miR-181c using antimiR-181c rescues diabetes-impaired angiogenesis and wound healing. We aim to test hypoxia-responsive copolymer nanoparticles (HRCNs) as a delivery system to target antimiR-181c to sites of ischaemia/wounding.

Methods and Results: We have synthesised and loaded antimiR-181c onto HRCNs comprised of a polyelectrolyte-lipid conjugate(PEI-DOPE), polyethylene glycol (PEG), and a hypoxia-sensitive azobenzene (Azo) linkage between the PEG and PEI-DOPE components. Transmission electron cryomicroscopy showed that the nanoparticles are 200 nm in size. Cell viability assays conducted in human coronary artery endothelial cells showed no evidence of cytotoxicity. Next, in a Matrigel tubulogenesis assay, antimiR-181c-HRCNs increased number of tubules (848±291), branches (754±79) and branching length (12601±603) under hypoxic and high glucose conditions compared to antimiR-Neg-HRCNs controls (number of tubules: 75±35; branches: 84±11; branching length: 7674±2350; $P<0.05$ for all). However, these effects were not seen in normoxia, highlighting the specificity of HRCNs. *In vivo*, in the diabetic murine wound healing model, antimiR-181c-HRCNs promoted wound closure (81±14%) compared to antimiR-Neg-HRCNs (46±18%, $P<0.0001$). These effects were seen as early at Day 2 post-wounding (antimiR-181c-HRCNs: 19±7% vs. antimiR-Neg-HRCNs: 6±4%, $P<0.05$).

Conclusions: HRCNs effectively deliver antimiR-181c to rescue diabetes-impaired angiogenesis. This presents antimiR-181c-HRCNs as a novel therapeutic strategy for the treatment of diabetic vascular complications.

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Genetic and biochemical association of the SLC22A3 transporter with Lp(a) levels and uptake in liver cells

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Lp(a) is an atherogenic lipoprotein formed by the binding of a low-density lipoprotein to apo(a). Elevated Lp(a) levels (>50 mg/dL) associate with cardiovascular diseases and are regulated by a large variable number tandem repeat (VNTR) in *LPA*. Variations in a neighbouring gene, *SLC22A3*, also associate with Lp(a) levels. In this study, we sequenced *LPA* and *SLC22A3* in 54 individuals with Lp(a) levels >50 mg/dL from a local cohort (Otago LPA, n=589) to identify common variants (MAF>0.01). These were tested for their association with Lp(a) levels in Otago LPA and a larger European cohort (GCKD, n=4930). Two linked variants in *SLC22A3*, rs1810126 and rs3088442, were associated with Lp(a) after adjusting for the *LPA* VNTR (Otago LPA, $P = 3.4 \times 10^{-5}$ and 2.2×10^{-5} and GCKD, $P = 1.6 \times 10^{-58}$ and 1.8×10^{-58}, respectively) with an allele effect of ~10 and 9 mg/dL for both variants in the LPA and GCKD cohorts. The rs3088442 variant residing in the 3′UTR associates with elevated Lp(a) and increased *SLC22A3* expression in Chinese. As SLC22A3 is known to transport molecules regulating macropinocytosis i.e. sodium and serotonin, and as Lp(a) is endocytosed by macropinocytosis, we tested whether overexpressing SLC22A3 influenced Lp(a) uptake. The overexpression of both wildtype SLC22A3 and a variant form of SLC22A3 (p.Thr44Met) associated with Lp(a) levels in Polynesians, significantly blocked Lp(a) uptake in liver cells. These results suggest that SLC22A3 regulates Lp(a) uptake via one of the many substrates it transports.

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The inflammatory profile of human monocyte-derived macrophages is associated with blood lipid levels

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Introduction: Unstable atherosclerotic plaques have a higher proportion of inflammatory (M1:CD86) compared to anti-inflammatory (M2:CD163) macrophages. While the plaque environment impacts macrophage phenotype, many macrophages are derived from blood monocytes which will have been exposed to blood lipids. Here, we investigated 1) whether the inflammatory state of monocyte-derived macrophages is associated with individuals' blood lipid profiles and 2) whether oxLDL directly induces inflammatory changes.

Method: Blood was collected from (n=20) human controls. Lipid levels were measured, and monocytes differentiated into macrophages. Macrophage inflammatory profile was assessed by CD86 and CD163 expression and cytokine production (TNF, IL-1β, and IL-6). In addition, monocytes were isolated from 6 normo-lipidaemic individuals and cultured with oxLDL prior to stimulation with LPS/IFNγ and assessment of cytokine production.

Results: Macrophage inflammatory phenotype was related to lipid levels. Positive correlations were found for CD86/CD163 with triglyceride/HDL-C, triglycerides and ApoB/ApoA1 (all $p<0.001$), Cholesterol/HDL ($p=0.01$), and ApoB ($p=0.02$). Functionally, macrophage TNF and IL-1b production positively correlated with oxLDL ($p=0.038$ and 0.015, respectively) and negatively with ApoA1 ($p=0.004$ and 0.02, respectively). Monocyte-derived-macrophages pre-exposed to oxLDL produced significantly higher IL-1β but lower IL-10 (in response to LPS/IFNγ), compared to non-oxLDL pre-exposed cells.

Discussion: Our findings suggest that the inflammatory phenotype adopted by macrophages in atherosclerotic plaques begins with priming in the circulation and may be directly induced by exposure to perturbed lipid levels, such as oxLDL levels. The increased inflammatory phenotype relative to triglyceride/HDL may partly explain the association of this ratio with atherosclerotic plaque instability.

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Gene expression profiling of myocardial ischaemic preconditioning and myocardial stunning

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Myocardial ischaemia caused by atherosclerosis is a common finding in the failing heart. Myocardial ischemic preconditioning (IPC), which is defined as brief episodes of ischaemia, protects the heart from subsequent prolonged ischemic injury, and reduces infarct size. Myocardial stunning refers to transient contractile dysfunction in the settings of ischaemia that recovers without permanent damage. Despite extensive research on IPC and myocardial stunning in the setting of ischaemia-reperfusion (I/R), transcriptional landscape of these phenomena remains unknown.

We established a novel rat model of I/R injury with and without IPC using open-chest left anterior descending artery occlusion-reperfusion technique in six-week-old male Sprague-Dawley rats. Echocardiography and 2,3,5-triphenyltetrazolium (TTC) staining was performed. RNA isolated from left ventricle tissue was subjected to RNA sequencing for gene expression changes. DNA methylation was assessed with Methyl-minor.

In the presence of IPC (2 cycles of 5min of I/R followed by 13.5min of ischemia), echocardiography acquisitions showed a profound myocardial stunning at 4h of reperfusion, which completely resolved at 48h. However, in the absence of IPC, we observed necrosis at 4h of reperfusion and sustained injury at 48h as assessed by TTC staining. The transcriptomic analysis identified genes differentially expressed in IPC (53 genes), stunning (1573 genes) and necrosis (1279 genes) when compared to control. Multiple novel genes were exclusively associated with IPC or stunning including *Apold1, Fgfr4* and Zfand2a. DNA methylation pattern identified that expression of these genes was regulated by DNA methylation indicating potential role of epigenetic mechanism in IPC and stunning.

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Selectively targeting the Gasdermin-D pore attenuates cardiac inflammation and fibrosis after ischemia reperfusion injury

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Inflammation plays a critical role in the clearance of cellular debris to promote tissue repair in cardiac injury pathophysiology. However, inadequately controlled inflammation contributes to adverse cardiac remodelling after acute myocardial infarction (AMI). This is driven by persistent activation of the NLRP3 inflammasome-Gasdermin-D (GSDMD) pathway with subsequent secretion of the inflammatory cytokine IL-1β. We investigated whether the FDA-approved therapeutic, Disulfiram, used to treat chronic alcoholism but recently shown to inhibit GSDMD, could reduce inflammation and thus improve ischemia/reperfusion (I/R)-mediated cardiac injury. Left coronary artery ligation was performed for 1h in 12-week-old C57BL6 mice, followed by reperfusion with or without 25/50mg of Disulfiram administered at reperfusion and daily until termination. At termination, (day7 and 28) cardiac function was measured by echocardiography, whilst fibrosis and inflammation were assessed by histology and RT-PCR. Flow cytometry assessed leukocyte populations in blood, spleen, bone marrow and heart. Control and Disulfiram-treated mouse BMDMs and human THP-1 cells were investigated for secreted inflammatory cytokines. Echocardiography showed significant improvements in ejection fraction after 50mg/kg Disulfiram, 7-days post-I/R injury($p<0.01$). Cardiac fibrosis and cardiac inflammatory and fibrosis gene expression was attenuated by Disulfiram D7 and D28 post-AMI (~$p<0.001$). This was associated with reduced inflammatory cell abundance in blood, spleen, bone marrow and heart. In LPS and ATP/Nigericin treated BMDMs and THP-1 cells, Disulfiram attenuated IL-1ß and IL-6 secretion ($p<0.001$). This study demonstrates that Disulfiram reduces inflammation by inhibiting IL-1ß secretion. Therefore, targeting the GSDMD pore may represent a novel way to provide cardio-protection post-AMI.

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Understanding the cellular and transcriptomic landscape of diabetes-associated peripheral artery disease by single-cell RNA sequencing.

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Peripheral artery disease (PAD) is characterised by reduced blood flow to the extremities due to occluded or narrowed arteries, which can lead to limb pain, non-healing wounds and gangrene necessitating surgical amputation. Diabetes is a common comorbidity that leads to worse clinical outcomes for patients. Our understanding of the contribution of diabetes to PAD pathogenesis is unclear. To gain greater insight into the molecular mechanisms and pathways underlying non-diabetes and diabetes-associated PAD, we conducted an unbiased transcriptomic investigation in mice. Gastrocnemius muscle of age-matched non-diabetic and 20-week diabetic *Apoe*$^{-/-}$ mice were harvested 14 days after hindlimb ischaemia for single cell RNA-sequencing. Clustering analysis identified 24 cell populations. Fibro-adipo-progenitor (FAP) and endothelial cells (ECs) contributed the largest proportion of cell numbers in muscle. Both were increased ~1.4-fold in response to ischaemia in non-diabetic mice, but the FAP response was impaired with diabetes. Computational modelling identified increased ligand-receptor interaction strength in both cell types with ischaemia. These were less pronounced with diabetes. Interestingly, FAP-EC collagen interactions were increased with ischaemia, and collagen-encoding genes were differentially expressed in FAPs in non-diabetic and diabetic animals. We validated our findings in amputated tissues from diabetes-associated PAD patients and identified ~3-fold increase in collagen IV content in ischaemic vs non-ischaemic areas, and importantly, collagen IV colocalized with capillaries in ischaemia. These findings suggest a role for FAP and EC interactions in diabetes-associated PAD and may provide insight into potential therapeutic targets for improved clinical outcomes for patients.

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Modulation of endothelial-to-mesenchymal transition by an epigenetic drug GSK-126 attenuates diabetes associated atherosclerosis

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Background. Endothelial to mesenchymal transition (EndMT) transforms endothelial cells into mesenchymal-like cells. Experimental data suggests EndMT may contribute to cardiovascular disease in diabetes. Epigenetic mechanisms are crucial in cardiovascular disease. Recently, expression of a histone methyl transferase, Enhancer of zest homolog 2 (EZH2) is observed to be elevated in EndMT in coronary artery disease. However, its role in EndMT in diabetes-associated atherosclerosis remains unclear.

Methods. EndMT was induced using high glucose (HG) ± TNF-α in human aortic endothelial cells (HAECs). GSK126 was used to inhibit the EZH2 methyltransferase activity. Additionally, we conducted RNA sequencing and EZH2 knockdown studies to validate EZH2 role in EndMT. Furthermore, we investigated the levels of EZH2-mediated-H3K27me3 and effect of GSK126 treatment on EndMT using a diabetic mouse model of atherosclerosis.

Results. EZH2-mediated-H3K27me3 in HAECs was elevated with TNF-α ± HG and blunted with GSK-126. Gene and protein expression changes also confirmed the induction of EndMT by HG ± TNF-α which was mitigated by GSK-126. Transcriptomic analysis revealed EZH2-mediated chromatin modifications regulated the expression of over two hundred genes implicated in EndMT with GSK-126 treatment restoring the expression of 76 important genes e.g., NOS3. EZH2 knockdown experiments in HAECs also provided additional confirmation of its involvement in EndMT. Immunofluorescence staining of the aortic endothelial layer in diabetic mice showed increased H3K27me3, accompanied by the colocalization of endothelial and mesenchymal markers which was blunted by GSK-126 treatment.

Conclusion. This study underscores the significant impact of inhibition of EZH2-mediated chromatin changes on EndMT progression in diabetes associated atherosclerosis.

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Therapeutic *Akkermansis muciniphila* supplementation to enhance atherosclerotic plaques stability in the tandem stenosis mouse model

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Atherosclerotic Cardiovascular diseases (ASCVDs) pose a significant global health challenge, representing a primary cause of mortality worldwide. Recent research has emphasised the role of the gut microbiome and found that *Akkermansia muciniphila*, a gut bacterium, can modulate the immune system, insulin resistance, blood glucose and atherosclerosis development. However, the effect on plaque stabilisation remains unknown.

In this study, both male and female ApoE$^{-/-}$ mice were fed a high-fat diet at 7 weeks of age and underwent tandem stenosis surgery at 13 weeks of age. After surgery, they were then treated with live *A. muciniphila* (AKK) or autoclaved dead *A. muciniphila* (DAKK) by daily oral gavage for 7 weeks. Analysis of microbiome sequencing data showed that AKK treatment reduced alpha diversity in the stomach compared to DAKK, indicating successful delivery of AKK. There are no significant changes in alpha diversity in stool samples. AKK treatment decreased circulating white blood cells, particularly CD8 T cells, CD4 T cells, CD45^{+} T cells, TCR^{+} T cells, and Neutrophils, with no significant changes in CD11c^{+} Dendritic cells. In terms of atherosclerosis plaque composition, AKK treatment reduced lipid content, intraplaque hemorrhage, and CD68 foam cells, suggesting enhanced plaque stabilisation. Other parameters, such as total cholesterol, triglyceride, LDL and HDL, remained unchanged. Plasma cytokine arrays showed AKK significantly increased MMP3. FITC dextran experiments suggested that AKK had the potential to seal the LPS-induced leaky gut. Together, this study suggests that AKK treatment may have beneficial effects on the digestive microbiome ecosystem and plaque stabilising effect in atherosclerosis.

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Atheroprotective effects of GLP-1 delivered by DNA minicircle

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BACKGROUND: GLP-1 receptor agonists have cardiovascular benefits, including a reduction in heart attacks, strokes and cardiovascular death. However, these agents remain difficult to use because of costly drug manufacture and ongoing delivery by repeated injections. To overcome these challenges, we have developed a simple way for the body to make GLP-1 and liberate it into the circulation from an intramuscular DNA mini circle. We now show that this strategy is also vasculoprotective.

METHODS: Apolipoprotein E KO mice made diabetic using streptozotocin, were randomised to receive a DNA minicircles (10ng) encoding A2G-GLP-1-27 (a DPP-4 resistant variant) or vehicle alone, 1 week later. After 10 weeks, the aortas were analysed to determine changes in atherosclerotic plaque area and gene expression. GLP-1 levels were measured by ELISA.

RESULTS: Injection with a DNA minicircle encoding GLP-1, increased circulating concentrations of GLP-1 compared to vehicle alone. This was associated with a reduction in the total atherosclerotic plaque area in diabetic mice, without significant improvement in glycemic control or the induction of weight loss. The expression of atherogenic genes was also reduced in the aortas of mice treated with a GLP-1 minicircle; including TNF-alpha, IL-1beta and NOX4.

CONCLUSION: While high-dose therapy with GLP-1RA is required for weight loss and glucose-lowering, these studies support the emerging concept that relatively smaller doses that continuously sustain an elevated systemic level can also have cardioprotective benefits. Minicircles are a safe and straightforward way to deliver atheroprotective GLP-1 without the need for costly manufacture or repeated injections.

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A novel immunometabolic therapy for diabetic cardiovascular complications

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Diabetes, a widespread worldwide health concern, is associated with a high risk of cardiovascular complications, in particular, atherosclerosis. Significant macrophage infiltration, oxidative stress, and inflammation are recognized as drivers of diabetic cardiovascular complications. The increasing prevalence and lack of effective treatments necessitate the development of targeted therapeutics. Itaconate, a metabolite derived from the TCA cycle, is highly upregulated during pro-inflammatory macrophage activation and exhibits anti-inflammatory and anti-oxidative properties via various mechanisms, including induction of the main regulator of oxidant stress namely the transcription factor Nrf2, and inhibition of the NLRP3 inflammasome. This study investigated the potential anti-inflammatory and anti-oxidative properties of Itaconate in activated macrophages, with the aim of extending these findings to *in vivo* models. Primary wildtype murine bone marrow-derived macrophages were pre-treated with 4-octyl Itaconate (4OI, a cell-permeable derivate) (12.5μM,18 μM, 25μM) before priming with lipopolysaccharide (17h; 0.1 μg/mL) and activating with ATP (4h; 1 mmol/L). Protein secretion was determined by ELISA, and gene expression changes were assessed by qRT-PCR. All statistical analyses were performed using GraphPad Prism, with $p<0.05$ considered statistically significant. Both IL-1β gene expression and secreted IL-1β protein levels were significantly attenuated by 4OI ($p<0.0001$ and $p<0.0001$ respectively). Furthermore, 4OI significantly reduced the expression of inflammatory genes MCP-1, IL-6, and ICAM-1 ($p<0.0001$). 4OI resulted in a significant increase in the expression of the antioxidant gene NQO1 ($p<0.0001$), an important indicator of transcriptional activation of Nrf2 by 4OI. These results strengthen Itaconate's therapeutic potential for targeting inflammation and oxidative stress in diabetic cardiovascular complications.

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Mechanotransduction in immune cells: The hidden driver in aortic stenosis

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Calcific aortic valve disease (CAVD) is an inflammatory process that causes progressive narrowing of the aortic valve, impeding cardiac output. While CAVD can be detected early, the only treatments currently available to prevent heart failure rely on expensive and invasive valve replacement procedures.

In early CAVD, transvalvular flow becomes turbulent, increasing velocity and accelerating disease progression. This suggests a feedback loop where altered shear stress drives chronic valvular inflammation through mechanotransduction, converting mechanical stress into biochemical signals.

To investigate this, we used a microfluidic model of aortic stenosis that simulates high shear conditions and the calcified valve environment. Our results show that high shear stress activates monocytes, upregulating inflammatory markers, and affecting their metabolism, differentiation, and foam cell formation. Phosphoproteomics and functional assays identified downstream mechanisms, which were validated in monocytes from severe aortic stenosis patients before and after valve implantation.

Given that aortic stenosis shares remarkable similarities with atherosclerosis, including the involvement of immune cells and chronic inflammation, our findings can be translated into atherosclerosis research. The insights gained from this study may provide a deeper understanding of the common pathways driving both conditions, potentially leading to novel therapeutic targets applicable across the spectrum of cardiovascular diseases.

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Resolving inflammation by targeting FPR2 protects against diabetes associated atherosclerosis.

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Introduction: Many diabetic patients are at high risk of developing vascular complications, mainly manifesting as atherosclerosis. Like other diabetic complications, a hallmark feature of atherosclerosis is unresolved inflammation. Most current approaches focus on risk factor reduction rather than targeting the underlying pathological processes; thus, new approaches are needed. One promising approach involves the use of endogenous molecules called Lipoxins to promote the resolution of inflammation. Herein, our focus was to determine the mechanisms by which Lipoxins protect against diabetes associated atherosclerosis (DAA).

Methods: *ApoE*$^{-/-}$ mice were rendered diabetic by streptozocin and after 10 weeks, they were treated with either Lipoxin A_4 or its analogues, CT4-43 and KG522, for a further 6 weeks. At the end of the study, aortae were collected for gene expression analysis, plaque staining and RNA-sequencing.

Results: Using Lipoxins as treatment significantly reduced plaque area in aortae of diabetic *ApoE*$^{-/-}$ mice compared to non-treated diabetic mice without affecting body weight, blood glucose levels, HbA1c or lipid levels. Markers of inflammation were significantly reduced with Lipoxin treatment, including IL-6 and MCP1. RNA-sequencing analysis identified a number of differentially expressed genes (DEG), including markers of macrophage infiltration (Lipa, Wfdc17 and C3ar1), which were reversed by Lipoxins. Increased expression of these genes, which are related to inflammation and cholesterol metabolic pathways, were also confirmed in diabetic mice aortae by qPCR.

Conclusion: Lipoxins have the potential to ameliorate DAA in *ApoE*$^{-/-}$ mice as measured by reduced aortic plaque area and a downregulation of genes relevant to atherosclerosis, independently of metabolic parameters.

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LIPOXIN MEDIATES RESOLUTION OF DIABETES-ASSOCIATED ATHEROSCLEROSIS (DAA) IN APOE$^{-/-}$ DIABETIC MICE VIA A DIRECT EFFECT ON PERIVASCULAR ADIPOSE TISSUE (PVAT)

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Background: Atherosclerosis in diabetes is driven by chronic inflammation due to hyperglycaemia, involving complex signalling pathways, potentially including "outside-in" signalling from perivascular adipose tissue (PVAT). This study was designed to investigate whether Lipoxin A4 and two mimetics (CT4-43, KG522) protect against atherosclerosis by acting on the PVAT.

Methods: Six-week-old ApoE$^{-/-}$ mice were made diabetic with streptozotocin. The study included a 10-week prevention arm, with LX treatment from the onset of diabetes, and a 16-week intervention arm, with LX treatment initiated after 10 weeks of diabetes for a further 6 weeks. At the end of the study, aortae and PVAT and plasma were collected for immunohistochemical, gene and RNA sequencing and metabolic analysis (n=30/group).

Results: LXs significantly reduced atherosclerotic plaque area ($p<0.0001$) in diabetic mice without affecting any metabolic or haemodynamic parameters. In the prevention arm, LXs regulated inflammatory, metabolic, and signalling molecules in the PVAT, while in the intervention arm, LXs primarily targeted metabolic pathways, insulin, AMPK signalling, and lipid metabolism. LXs significantly downregulated ($p<0.001$) inflammatory genes (e.g., *IL-6, IL1-β, MCP-1, TNF-α, NFκB, RANTES, ICAM, VCAM*) and macrophage markers, and restored metabolic regulation (e.g., *INSR-1, GLUT4, AMPK, UCP-1, AdipoQ, leptin*) in the prevention study. Complementary in vitro studies revealed no protective effects of LXs against TNF-α or IL1-β, but demonstrated an effect on lipid droplet accumulation in 3T3-L1 adipocytes.

Conclusion: LXs protect against atherosclerosis by modulating PVAT inflammation and enhancing metabolic activity. Further studies in type 2 diabetes and obesity models are warranted to inform future clinical trials.

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Development of a 3D-printed tandem stenosis model for consistent induction of plaque instability in *ApoE*$^{-/-}$ mice

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Tandem stenosis (TS) is a surgical technique that creates two stenoses on the carotid artery, leading to low shear stress and high tensile stress. This environment promotes unstable plaque development, characterized by neovascularization, thin-cap fibroatheroma, active inflammation, outward remodelling, and plaque rupture, making TS an ideal preclinical model for drug testing.

We conducted over 3,000 TS surgeries and trained scientists across ten labs. Our studies showed around 50% intraplaque haemorrhage, while others reported a range of 25% to 80%. We found that consistent flow reduction following TS, relies on precise suture tightness and needle placement, typically monitored by a vascular ultrasonics flow probe- an equipment not available in every lab. This surgical precision is crucial, as it can cause variability in the induction of shear stress-dependent plaque phenotypes.

To address these limitations, we have developed several prototypes of the 3D-printed TS model with biocompatible resin. These models are specifically designed to enhance 1) peripheral neovascularization or 2) intraplaque haemorrhage, achieving these outcomes within 6 weeks post-implantation (n=18). We found that the 3D-printed TS generated a similar flow reduction (58%±9%) compared to the traditional TS (54%±5%). The overall plaque burden in the aortic sinus and carotid arteries showed no significant differences. Plaque stability markers such as necrotic core, collagen, lipid, CD31, CD68, and Ter-119 also showed no significant differences between them. Our novel approach offers a more standardized, and easily applicable method for inducing plaque instability, improving the reliability of this model for future drug testing and mechanistic studies.

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Trimethylamine-N-Oxide's role in atherosclerotic plaque stability: Insights from fluoromethylcholine studies in a tandem stenosis mouse model

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Trimethylamine-N-Oxide (TMAO) is a metabolite linked to the prognosis of atherosclerotic vascular diseases while fluoromethylcholine (FMC) is a lyase inhibitor for gut microbe TMA metabolism, that prevents TMAO generation. This study investigates the relationship between TMAO levels and atherosclerotic plaque stability using tandem stenosis (TS) mice, a model for unstable plaques.

Eighteen 7-week-old ApoE$^{-/-}$ mice were fed a high-fat diet for 6 weeks. At 13 weeks, the mice underwent carotid artery TS surgery and were randomly fed either high-choline (3% choline, n=8) or high choline with FMC (n=10) diet and then sacrificed at 20 weeks for further analysis. TS segments were immunostained to compare mice with varying TMAO levels. Gut permeability was assessed using FITC-Dextran, plasma cytokines via Cytometric Bead Array, and stool samples were microbially profiled using MiSeq.

FMC significantly reduced plasma TMAO levels ($p<0.005$). Hemostatic analysis showed no statistical difference between the groups in blood components and plasma lipids. Aortic sinus staining showed no difference in plaque burden or composition (lipid, collagen, CD31, CD68, smooth muscle cells). However, FMC-treated TS segment plaques displayed increased CD68 and SMA-alpha levels and decreased TER119 ($p<0.05$), indicating changes in intraplaque hemorrhage markers. Gut microbiota alpha diversity was significantly reduced after FMC treatment.

FMC effectively reduces plasma TMAO and alters gut microbiota alpha diversity. Although no correlation was found between TMAO levels and plaque burden in the aortic sinus, TS segment plaques showed phenotypic changes with FMC treatment, indicating potential plaque-stabilizing effects through coagulation pathways, suggesting its therapeutic potential in atherosclerotic cardiovascular diseases.

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In situ measurement of live human plaque permeability and cell infiltration using spectral photon counting CT imaging

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The imaging of arterial plaques presents a significant challenge due to the small thickness (1-4 mm) and low X-ray attenuation of the tissue compared to the surrounding artery. Examination of key events and general properties of plaques can be difficult to measure. Using X-ray spectral photo counting CT (SPC-CT) imaging, we demonstrate fundamental physiological properties of atherosclerotic plaques.

Plaques from the carotid artery of stroke patients were obtained via carotid endarterectomy surgery and transfer to the laboratory on ice before being cut into 3 mm thick rings and placed in tissue culture media. Plaque tissue viability was demonstrated by the release of lactate into the media. Plaque sections were imaged using MARS small bore SPC-CT scanner taking 1440 flat fields per rotation at energy bins of 18-26, 26-36, 36-46, 56-118 KeV. Material decommission images were generated using MARS vision software with energy attenuation calibration with phantoms of known composition.

Incubation of live plaque with 20 mM NaI showed iodide ions rapid diffused through the plaque within an hour of exposure. Similarly, cold iodine labelled low density lipoprotein could rapidly diffuse through the plaque tissue.

Human monocytes can be labelled for spectral imaging as they readily take up gold nanoparticles. Live human plaques sections were incubated with nanogold labelled monocytes before SPC-CT X-ray imaging which showed an infiltration of 0.5×10^6 monocytes per plaque segment within the first 12 hours of incubation.

SPC-CT imaging is able to demonstrate key physiological process occurring within atherosclerotic plaques while preserving tissue integrity.

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The utility of Computed Tomography Coronary Angiography for predicting major adverse cardiac events in patients undergoing investigation for chest pain: a single-centre study.

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Background: The strength of CT coronary angiography (CTCA) is in ruling out significant coronary artery disease (CAD) in symptomatic intermediate risk patients. CTCA is gaining attention as a tool for stratifying patients' risk of major adverse cardiac events (MACE), defined as a composite of all-cause mortality, myocardial infarction and stroke or transient ischaemic attack. This study evaluated the ability of stenosis reporting on CTCA to predict MACE in patients undergoing investigation for stable chest pain at Townsville University Hospital.

Methods and Results: 1003 patients who underwent a CTCA between January 2015 and November 2023 were followed up until February 2024. For each patient, maximum degree of stenosis on CTCA, coronary artery calcium score (CACS) and cardiac risk factors were collected. 471 patients had no stenosis on CTCA, 181 had 1-49% stenosis, 237 had 50-69% stenosis and 114 had >70% stenosis. 116 patients had ICA performed of which 29 had a subsequent PCI and 9 had a CABG. The hazard ratio for suffering a MACE was 3.74 in patients with 70% or more stenosis on CTCA compared to the 0% stenosis group. Adjusted cox regression analysis found that whether stenosis reporting on CTCA provides incremental benefit beyond CACS for predicting MACE depends on the definition of MACE. ROC curve analysis revealed that the performance of CTCA was better in women than men and in Aboriginal and/or Torres Strait Islander patients than other Australians although these results were not statistically significant.

Conclusions: Maximum degree of stenosis on CTCA can predict MACE.

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A new mathematical model for plaque growth

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Agent-based models (ABMs) represent cells as individual entities that move and interact with each other and with cytokines and lipids. These are in contrast to differential equation models that model either entire populations of cells or cell densities. We present a new ABM that models macrophage interactions in early plaque.

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A mathematical model for the role of smooth muscle cells phenotype switching in atherosclerotic plaques

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Vascular smooth muscle cells (SMCs) play a fundamental role in the pathophysiology of atherosclerosis. SMCs form a cap over the mid-stage atherosclerotic plaque, and they may ingest lipids similar to plaque macrophages. This stimulates a switch, cells triggered by internal lipid accumulation, of SMCs to a macrophage-like phenotype. However, these SMC-derived macrophages (SDMs) are ineffective in clearing lipids and apoptotic cells from the lesion microenvironment, and they have a reduced phagocytic capacity compared with classical immune cells. Failure to remove lipids and apoptotic cells from the atherosclerotic plaque leads to secondary necrosis and an inflammatory necrotic core. The stability of the fibrous cap, which is essential for plaque maintenance, is directly related to the number of SMCs it contains. A thin fibrous cap with fewer SMCs increases the risk of plaque rupture, potentially leading to thrombosis and clinical complications such as heart attack and stroke. Despite its importance, the mechanisms underlying phenotypic switching, cap formation, and plaque stability are not fully understood. This study proposes a simple mathematical model to examine the role of SMC phenotypic switching in atherosclerotic plaque progression. The model considers different cell populations and their lipid content, as this lipid load influences SMC phenotypic switching. Through analysis and numerical simulations, the study explores the balance between SMC and macrophage populations during phenotypic switching. Sensitivity analysis identifies key biological parameters associated with plaque stability. These insights contribute to a better understanding of the factors and mechanisms driving SMC phenotypic switching in atherosclerosis progression and stability.

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Triglycerides contribute to the pro-inflammatory actions of neutrophil extracellular traps

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Background: Neutrophils are the most abundant white cells in blood and mediate innate immune functions via oxidative burst, phagocytosis and secretion of neutrophil extracellular traps (NETs). NETs, an extracellular network of decondensed chromatin and proteins, form aggregates with platelets and stimulate release of pro-inflammatory cytokines from endothelial cells, macrophages, and lymphocytes. Coronary NET burden is a predictor of myocardial infarct size and is associated with all-cause mortality. However, to date no study as examined if NETs contain lipids.

Objective: Identify NET associated lipids and determine their functional activity.

Methods: Neutrophils were obtained from healthy volunteers, activated with PMA and NETs isolated by partial restriction enzyme digestion. Lipids were extracted using the Folch extraction method and identified by LC/MS. NETosis, oxidative burst and chromatin swelling were analysed in neutrophils treated with inhibitors of the triglyceride synthesis enzymes DGAT1 and DGAT2. The impact of triglyceride synthesis inhibition on NET-mediated macrophage polarisation and thrombosis was assessed.

Results: NETs contained a wide variety of lipids including triglycerides (32.02±10.83%, of total lipid content vs 4.23±2.21% in whole cells). NET formation was inhibited by pretreatment with inhibitors of both DGAT1 and DGAT2, but not individually. Inhibition of triglyceride synthesis decreased oxidative burst and chromatin swelling. Pro-inflammatory macrophage CD64 expression and IL-1beta secretion were lower in cells treated with NETs lacking triglyceride compared to control NETs. NET-dependent thrombosis was decreased by triglyceride synthesis inhibition.

Conclusion: The triglyceride content of NETs is an important modulator of inflammation which could be a target for therapeutic intervention.

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Elevated lipoprotein(a) and familial hypercholesterolaemia in the coronary care unit at Sir Charles Gairdner Hospital

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Background: Familial hypercholesterolaemia (FH) and elevated lipoprotein(a) [Lp(a)] are heritable risk factors for coronary artery disease (CAD). Universal screening in patients presenting to coronary care units (CCU) represents a unique opportunity to detect new cases.

Aim: This study aims to examine the prevalence of FH and elevated Lp(a) in patients admitted to CCU at Sir Charles Gairdner Hospital (SCGH), and to assess the incidence and outcome of lipid clinic referral.

Method: This prospective cohort study included 138 patients admitted to CCU with acute coronary syndrome (ACS) over 4 months in 2023. Patient demographics, cardiovascular risk factors, medications and family history were collected. Lipid profiles and Lp(a) levels were sent during admission. A Dutch Lipid Clinic Network Score was calculated using corrected LDL and a value ≥ 6 was considered diagnostic of FH. Lipid clinic referrals and genetic testing were recorded.

Results: The frequency of elevated Lp(a) was 34.7%. There was no significant difference in frequency of elevated Lp(a) in patients with and without premature CAD ($p = 0.18684$). The frequency of FH in patients presenting to CCU was 8.7%. Patients with FH were significantly more likely to have premature CAD ($p = <0.05$). 6 patients were referred to lipid clinic, 2 of whom had phenotypic FH.

Conclusion: The results support routine screening for FH and elevated Lp(a) in patients admitted to CCU for ACS. Optimisation of screening and referral practices should be considered to enable prompt detection and access to cascade screening.

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Low-density lipoprotein cholesterol estimation in youth: Sampson equation superior in predicting mid-adult carotid plaque

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Background: Accurate estimation of youth low-density lipoprotein-cholesterol (LDL-C) is essential for preventing atherosclerotic cardiovascular disease (ASCVD).

Objective: To compare the association between different LDL-C estimation equations and their discordance groups in youth and the presence of carotid plaque in mid-adulthood.

Methods: This study included 2058 participants from the Young Finns Study with fasting blood samples in youth (aged 3-18 years) and carotid ultrasound data in mid-adulthood (41-56 years). LDL-C was estimated using the Friedewald, Martin-Hopkins, Sampson, and DeLong equations—different mathematical formulations using the same inputs. Discordance was defined as differing LDL-C categorizations (acceptable <110 mg/dL versus dyslipidemia ≥110 mg/dL) between equations.

Results: Youth LDL-C dyslipidemia (versus acceptable) was consistently associated with carotid plaque across equations, with relative risks (RR) ranging from 1.36 to 1.42. Participants with acceptable LDL-C by the Friedewald equation but dyslipidemia by the Martin-Hopkins, Sampson, or DeLong equations had an increased risk of carotid plaque compared to those with concordant LDL-C classifications: Martin-Hopkins [RR (95% confidence interval) 2.44 (2.12-2.80)], Sampson [1.58 (1.17-2.14)], and DeLong [1.42 (1.10-1.83)]. Similarly, discordance between acceptable Martin-Hopkins LDL-C but dyslipidemia by Sampson or DeLong equations indicated an increased risk. No increased risk was observed in youth with acceptable Sampson LDL-C but dyslipidemia by the DeLong equation.

Conclusions: The Sampson equation provides the most accurate assessment of long-term risk of carotid plaque, outperforming the underestimation by Friedewald and Martin/Hopkins and the overestimation by DeLong. Using the Sampson equation for youth LDL-C estimation could improve early detection of future ASCVD risk.

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The functionality of high-density lipoproteins is impaired in patients with diabetes that have undergone toe amputations.

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Background: Circulating high-density lipoprotein cholesterol (HDL) levels have an inverse association with cardiovascular disease risk and risk of amputation in patients with diabetes-related foot ulcers. HDL functionality is impaired in patients with coronary artery disease, emerging as an improved predictor of disease outcomes. However, the role of HDL functionality in patients with diabetes-related amputations remains unknown.

Methods: Thirty patients with diabetes and eleven without diabetes undergoing toe amputations were enrolled in the study. Blood was collected at the time of surgery, 1-month and 6-months post-amputation, and from 20 healthy gender- and age-matched control participants. HDL was isolated from plasma and assessed for functionality of cholesterol efflux from macrophages, and anti-inflammatory and pro-angiogenic effects in endothelial cells.

Results: The cholesterol efflux capacity of diabetic HDL was reduced at 1-month post-amputation compared to non-diabetic HDL (-57%, $P<0.05$). In response to inflammatory stimulus, cells treated with diabetic HDL had elevated levels of *CCL2* (+49%) and *VCAM1* (+67%) at baseline compared to non-diabetic HDL ($P<0.05$, for both). *CCL2* increased linearly in cells treated with diabetic HDL up to 6-months ($P<0.05$), demonstrating exacerbation of inflammatory responses over time. The capacity of cells to form tubules reduced linearly over time by diabetic HDL ($P<0.05$). This was concomitant with reduced *VEGFA* gene expression at baseline compared to non-diabetic HDL (-47%, $P<0.05$), suggesting baseline impairment of inflammatory and angiogenic mediators has long-term impact on HDL functionality.

Conclusion: The cholesterol efflux capacity, anti-inflammatory properties and pro-angiogenic effects of HDL are impaired in patients with diabetes who had toe amputations.

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Diabetes induces TET2 dysfunction in bone marrow haematopoietic stem cells and accelerates DNMT3A R878H/+ clonal haematopoiesis in mice.

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Clonal haematopoiesis (CH), a blood disorder driven by somatic mutations in haematopoietic stem and progenitor cells (HSPCs), accelerates atherosclerotic cardiovascular disease (ACVD). Alarmingly, the mechanisms that underpin CH-driven ACVD are still largely unknown, and consequently there are no therapeutic interventions. Interestingly, CH is overrepresented in people with diabetes. The two most common "loss of function" genetic mutations in CH are found in DNMT3A and TET2. While loss of function mutations in either DNMT3A or TET2 alone has relatively little effect, the combined deficiency of these genes results in a myeloproliferative disorder. Recently, diabetes was shown to induce TET2 dysfunction via dysregulation of AMPK in mice. In this study, we explored if diabetes induced dysregulation of the AMPK-TET2 axis in bone marrow (BM) HSPCs to exacerbate murine *Dnmt3a*-driven CHIP. The most common mutation in CH is the heterozygous DNMT3A *R882H* mutation *(R878H mouse* equivalent). Using gold-standard animal models to mimic human CH (10% *Dnmt3a*$^{R878H/+}$ cells) we discovered that diabetes exacerbates *Dnmt3a*$^{R878H/+}$ myeloid expansion. BM HSPCs from mutant *Dnmt3a*$^{R878H/+}$ diabetic mice exhibited AMPK-driven TET2 dysfunction, resulting in HSPC hyperproliferation and myeloid skewing of mutant HSPCs. Excitingly, HSPCs TET2 dysfunction and clonal outgrowth was reversed by the AMPK activator, O-304, in diabetic mice with CH. Taken together, our findings indicate that hyperglycaemia is a risk factor for rapid clonal outgrowth in *Dnmt3a*$^{R878H/+}$ CH. Furthermore, our work suggests that individuals with diabetes and *Dnmt3a*$^{R878H/+}$ CH should be screened more frequently for CH complications such as ACVD and would benefit from AMPK agonists.

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Differential gene expression patterns during apoptotic and necroptotic cell clearance by primary macrophages

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Introduction: Enhanced programmed cell death (e.g. apoptosis, necroptosis) and defective efferocytosis are hallmark characteristics of advanced atherosclerosis. Macrophage efferocytosis of apoptotic cells becomes defective in advanced disease, resulting in secondary necrosis. Recent studies have described rapid transcriptional regulation during macrophage efferocytosis of apoptotic cells. However, the transcriptional regulation in macrophages during efferocytosis of necroptotic cells is unknown - the aim of this project.

Methods: We stimulated bone marrow-derived macrophages (BMDMs) with apoptotic or necroptotic cells (at a 1:10, macrophage: dead cell ratio) for 6-hours. Using QuantSeq 3' mRNA-seq kit, RNA-seq was performed, followed by differential gene expression analysis using DESeq2 (v1.32.0) in R (v4.1.1).

Results: BMDMs engulf necroptotic cells at higher ratios (1:10; $p<0.05$) but not at lower density of necroptotic cells (1:3 ratio). Compared to control cells, RNA-Seq show 119 and 110 genes were differentially regulated in macrophages exposed to apoptotic cells or necroptotic cells respectively, with 67 genes uniquely upregulated during efferocytosis of necroptotic cells. GO pathway analysis indicate the upregulation of specific biological processes during efferocytosis of apoptotic cells. These include apoptotic cell clearance (e.g. *Ccl2, Mertk, Nrh13,* and *Rac1)*, validating our efferocytosis model. Some distinct biological processes were upregulated in macrophages exposed to necroptotic cells, including positive regulation of pattern recognition receptor signalling (e.g. *Hspa1a*, *Rsad2*) and defense response (*Adam17, Ccl5, and Cxcl10*). We're currently validating these genes in BMDMs using siRNA.

Conclusions: Macrophages engulfing necroptotic cells indeed regulate unique transcriptional signatures, presenting an opportunity for targeted mRNA or siRNA therapies to treat atherosclerosis.

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PCSK9 AAV delivery to induce hypercholesterolemia in the tandem stenosis mouse model of plaque instability

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Unstable plaque is a major driver of cardiovascular events, imposing a significant global health burden. Current atherosclerotic mouse models are limited by poor disease mimicry and inconsistent plaque progression. Unstable plaque formation that resembles human pathology can be induced by tandem stenosis surgery, but is reliant on hypercholesterolemic Apolipoprotein E$^{-/-}$ (ApoE$^{-/-}$) mice fed a high-fat diet (HFD), limiting its broader application. This study aims to induce unstable plaque formation in wildtype mice treated with a liver-directed adeno-associated virus (AAV) to overexpress proprotein convertase subtilisin/kexin type 9 (PCSK9) and HFD. Male and female mice received PCSK9 AAV (0.5×10^{11} viral genomes, *i.v.*) or vehicle and were placed on HFD, alongside ApoE$^{-/-}$ and wildtype controls. After 7 weeks, two stenoses were created on their right carotid artery, and the HFD continued for 6 weeks, before plasma, carotid arteries and aortae were collected for analysis. PCSK9 AAV-treatment significantly increased circulating cholesterol compared to controls ($P < 0.01$, n = 4-8), and in male mice, cholesterol was higher than in ApoE$^{-/-}$ mice ($P < 0.01$, n = 4-5). Aortic plaque was also present in PCSK9 AAV-treated and ApoE$^{-/-}$ mice to a similar extent and totally absent in controls ($P \leq 0.001$, n = 5-9). Tandem stenosis induced unstable plaque formation in PCSK9 AAV-treated and ApoE$^{-/-}$ mice, which exhibited comparable fibrous cap thickness and CD68^{+} cell infiltration ($P > 0.05$, n = 4-5). Therefore, induction of hypercholesterolemia by PCSK9 overexpression in the liver and HFD allows for broader applicability of the tandem stenosis mouse model of unstable plaque.