Scientific Meeting

Australian Physiological Society

Australian New Zealand Society for Sarcopenia & Frailty Research

Melbourne - Victoria - November 2023

26 - 29 November 2023

HOSTED BY

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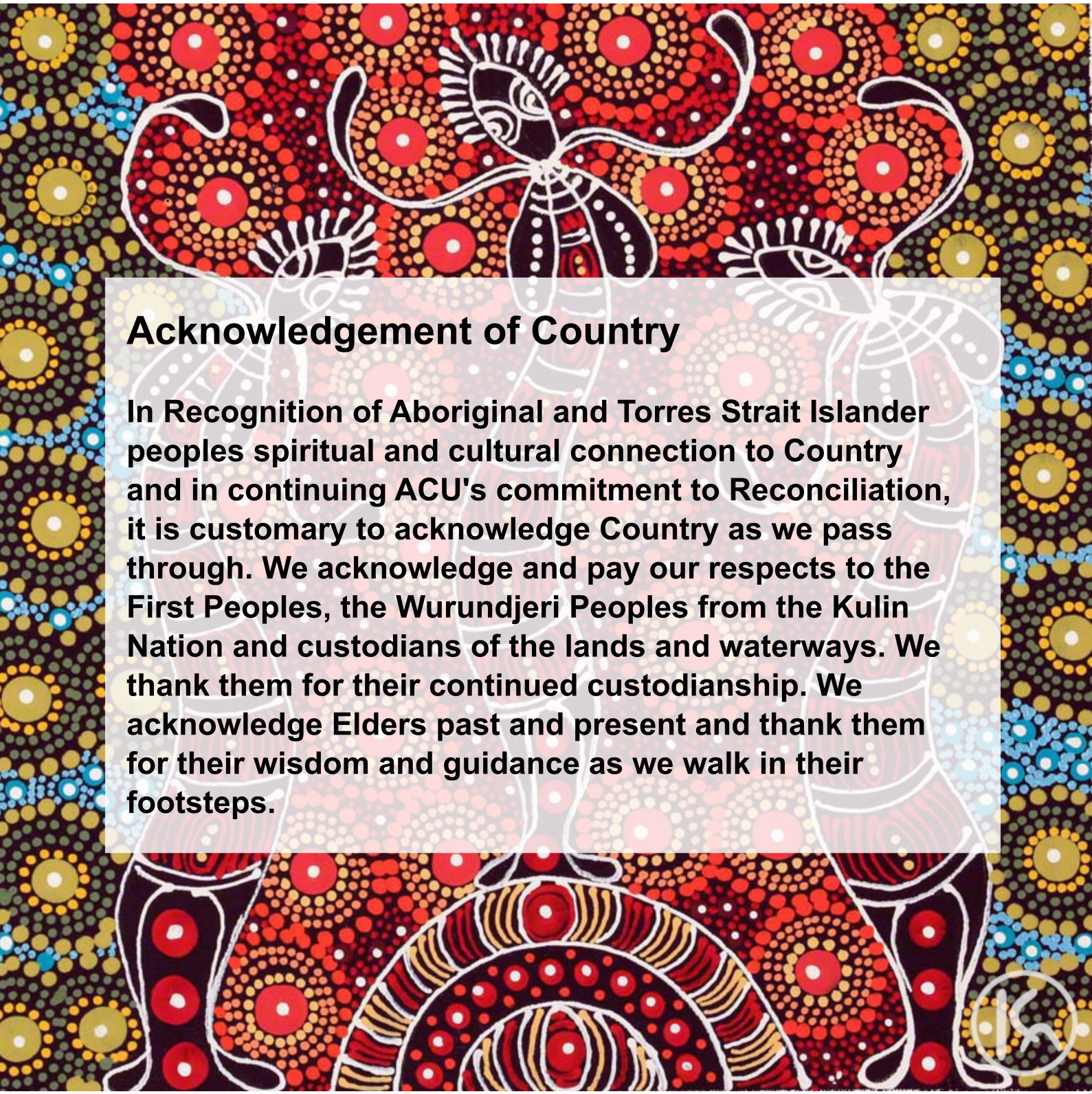
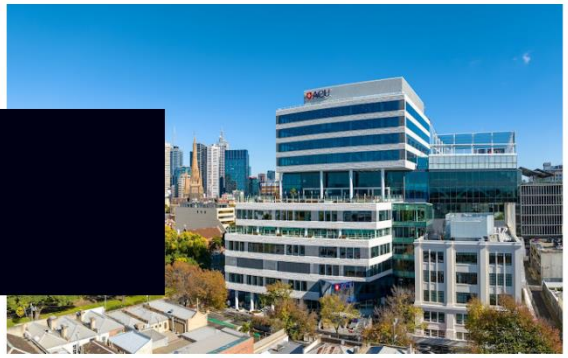


Acknowledgement of Country

In Recognition of Aboriginal and Torres Strait Islander peoples spiritual and cultural connection to Country and in continuing ACU's commitment to Reconciliation, it is customary to acknowledge Country as we pass through. We acknowledge and pay our respects to the First Peoples, the Wurundjeri Peoples from the Kulin Nation and custodians of the lands and waterways. We thank them for their continued custodianship. We acknowledge Elders past and present and thank them for their wisdom and guidance as we walk in their footsteps.

Welcome



It is with great pleasure that we welcome you to Melbourne and the Australian Catholic University for the 2023 AuPS | ANZSSFR scientific meeting.

We have an outstanding scientific program on offer with invited lectures, and three separate streams featuring nine AuPS symposia, four co-badged ANZSSFR themes, eight free communication sessions over fifteen posters. Complementing the program are the Welcome reception at Ted Exell Terrace *and* the conference dinner at *The Plaza Ballroom,* providing a chance to socialise while enjoying expansive views of our beautiful city and indulging in some of the finest local produce.

This year's conference sees an expansion of the traditional program with a number of annual initiatives. A key focus of both Societies is the support and development of our student and ECR members. On Sunday our ECRs will hold their traditional workshop and on Monday the famous mixer, and our dedicated student and ECR prizes and awards recognizing the achievements of our up-and-coming researchers.

We have experienced growing engagement with physiology education over the past years, and this demand has resulted in a dedicated education stream within the conference. This will run as a hybrid model facilitating the participation of academics from across the country, and includes workshops, prize lectures, a symposium and free communication sessions, a hands-on lab workshop and networking session.

Finally, the meeting provides the chance to celebrate our most talented researchers and educators with the awarding of the AK McIntyre and Michael Roberts Prize, Publication and Presentation prizes. Winners will be announced during the conference dinner and AGM.

We hope you enjoy the conference, and also get to experience all the beauty and liveliness that Melbourne has to offer.

Prof Nir Eynon (ARMI, Monash University) and Dr Nolan Hoffman (Australian Catholic University)
Conference Co-Chairs

Local Organising Committee: Co-Chair Prof Nir Eynon, Co-Chair Dr Nolan Hoffman, Dr Patrice Jones, Dr Alannah McKay, Dr Mehdi Belhaj, Dr Macsue Jacques and Dr Elizabeth Reisman

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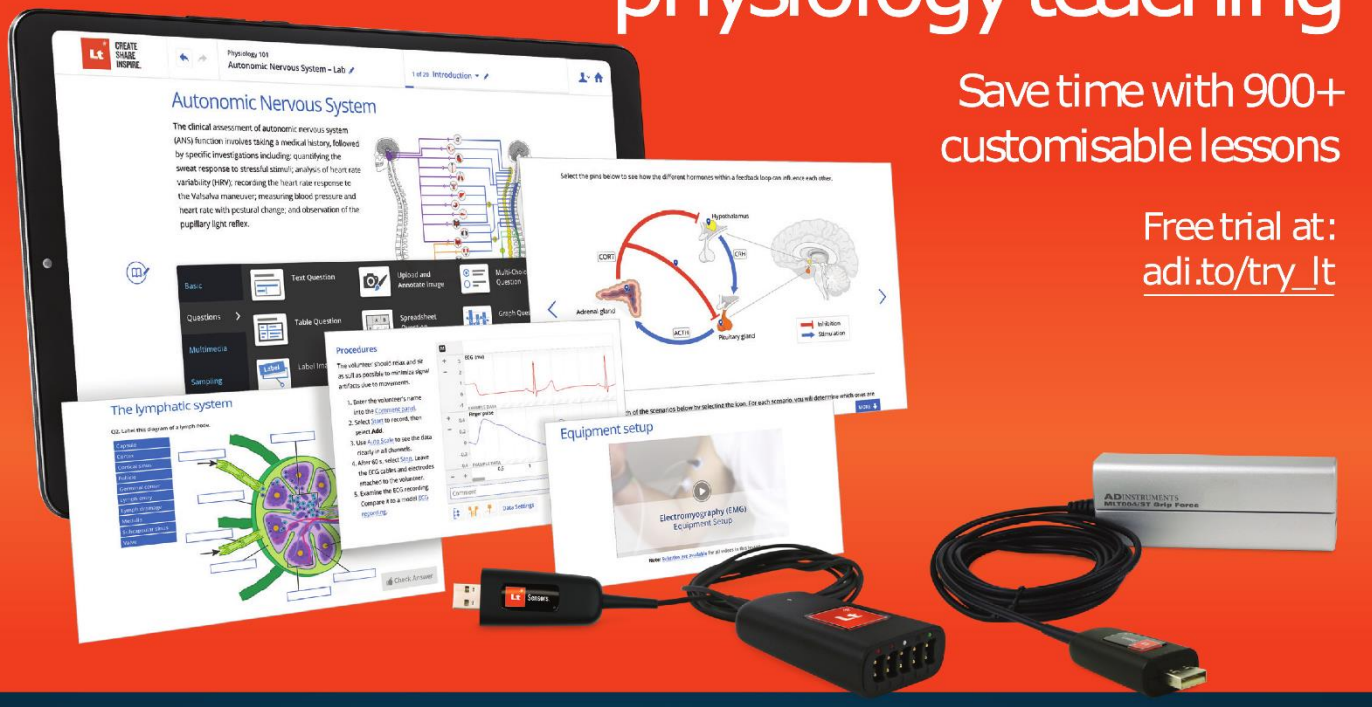

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For more information:

Lt + Lt Sensors: Parimal Kacha p.kacha@adinstruments.com
Langendorff: Amy Cheah a.cheah@adinstruments.com

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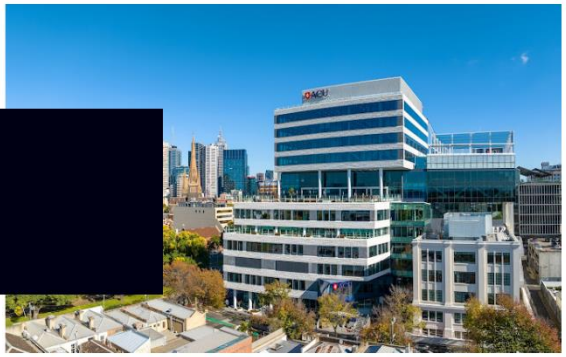
The Venue

Saint Teresa of Kolkata Building
ACU Building 421



Welcome/Registration Desk

Registration opens on Sunday 26/11 at 2pm for all conference delegates.

The Welcome/registration desk will be staffed each morning on Monday, Tuesday and Wednesday.

If you require assistance outside of these times, ACU staff and students and members of the LOC will be available throughout the event and can be identified by their ACU and AuPS lanyard.

Name Badges

Please wear your name badge at all times, as it is your entry into all sessions and enables security to identify you as a conference delegate.

Security

If you require any assistance or first aid during the event, security are located on ground floor near the concierge desk in the Mary Glowrey Building (building 420 next to the conference building).

If there is an Emergency, please contact police, fire or ambulance on 000, and campus security on 0477752510.

IT services

If you require IT support during the conference you can visit the IT services office located next to the security desk or from an ACU phone via extension 7272, option 1, option 5.

You may access Wi-Fi via Eduroam, or if registered for ACU Guest Wi-Fi. IT services will send your login credentials via text message.

Presenters

If you are presenting a talk or poster at the conference, please check your email for instructions.

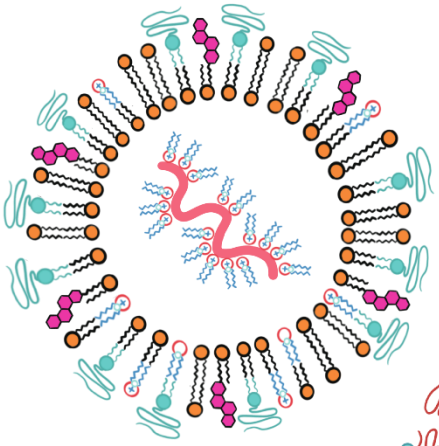
If you are presenting an oral talk, please save your talk on an external hard-drive and bring it with you to the allocated room at least 15 minutes prior to the session.

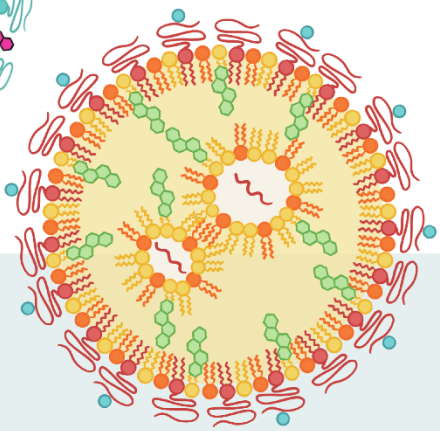
If you require assistance during the conference, please visit the registration desk, find an ACU staff member, or contact the Chair of your session.

VectorBuilder

mRNA Gene Delivery Solutions

mRNA possesses unique merits compared to other biologics and is a promising candidate for development and use as a drug. VectorBuilder provides a one-stop solution for the development of mRNA-based therapeutics such as vaccines, gene editing, chimeric antigen receptor, and protein expression in cells or embryos. Based on extensive design and production experience, our team can support researchers from in vitro transcription vector design and codon optimization, all the way to manufacturing of mRNA and LNPs for in vivo use.

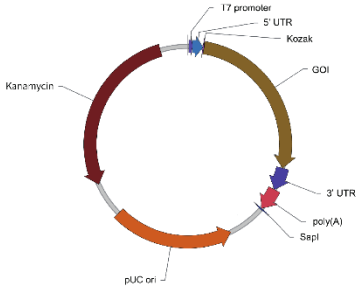




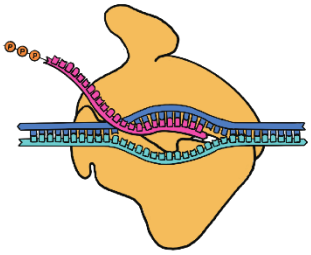
Highlights

- Custom IVT vector cloning with rapid turnaround.
- Variety of in-house validated 5' & 3' UTRs and polyA tails available for sourcing.
- T7 RNA polymerase-based synthesis for conventional and self-amplifying mRNA of up to 10,000 nt from ug to hundreds of mg scale.
- Modified nucleotides m1Ψ and m5C can be incorporated during synthesis to enhance mRNA translation and immune evasion.
- High-quality LNP-mRNA encapsulation at mg scale.
- Comprehensive quality controls and LNP profiling.
- We offer clinically oriented CRO services to assess LNP-mRNA gene delivery efficacy and safety using animal models including rodents and NHPs.

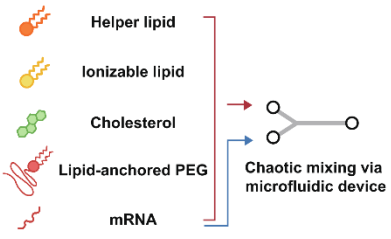
Our Capabilities



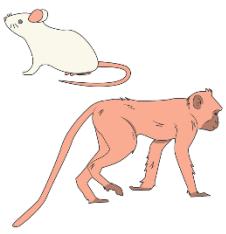
Custom In Vitro Transcription Vector Design



High Yield In Vitro Transcription and Capping



LNP Encapsulation Services



In Vivo Expression Testing

Floor Plan

ACU
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Melbourne Campus (St Patrick's)
115 Victoria Parade, Fitzroy, VIC 3065

402	St Mary of the Cross Square
403	The Daniel Mannix Building
404	Christ & Mercy Lecture Theatres
405	Cathedral Hall
406	Drake House
407	Art Gallery
408	Recital Hall
409	Visual Arts Building
411	32 Brunswick Street
412	Arts Precinct
413	38-40 Brunswick Street (Media Hub)
420	The Mary Glowrey Building (115 Victoria Parade)
421	Saint Teresa of Kolkata Building
422	Multi-level Carpark

KEY

- Food
- Cafe
- Male toilet
- Female toilet
- Accessible toilets
- Bike racks
- Elevator
- Locker
- Games area
- Accessible ramp
- Assembly point
- Automatic External Defibrillator (AED)
- First aid
- The School Locker campus store
- Post box
- Bus station
- Tram station
- Entry
- General parking
- Staf parking
- Accessible parking
- ACU Sport Gym

The University respectfully acknowledges the Wurundjeri people as the traditional custodians of the land on which this campus is situated.

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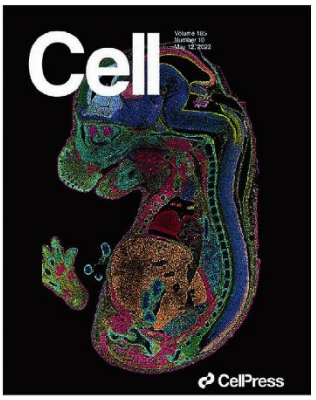
STOmics Stereo-seq



- The only spatial technology with no capital equipment required – use your existing histology platforms
- Highest resolution and largest tissue areas available
- The highest quality data available yet in spatial transcriptomics

AuPS2023 Starter Kit – ½ price starter kits available in 1x1cm, 1x2cm, 2x2cm & 2x3cm sizes

DNBelab-C4 scRNA

- No capital equipment required -
- reusable droplet generator included
- No Waste – each sample runs independently
- Very high capture rates with MGI's unique dual bead technology
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- **AuPS2023 Starter Kit** – free 16 sample scRNA kit when you order
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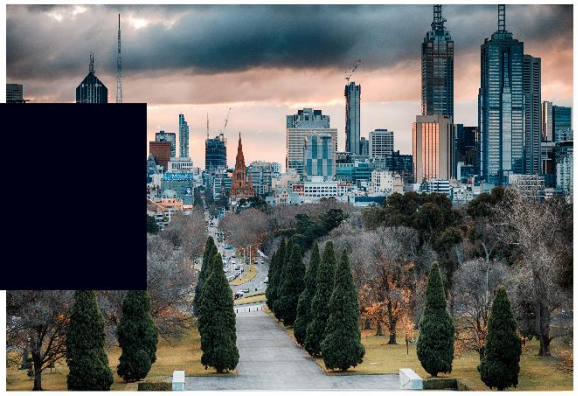
DNBelab C-TaiM 4RS cell isolator for scRNA & ATAC-seq

- Precise & compact droplet generator for only $29,400
- No Waste with 1 to 4 sample flexibility
- Runs both scRNA and ATAC-seq
- **AuPS2023 Starter Kit** – comes with a free 16 sample scRNA or ATAC-seq kit

Program Highlights



AuPS Invited Lecture

"Muscle membranes talk"

Prof Bradley S. Launikonis

5:15pm Sunday

Prof Bradley S. Launikonis

University of Queensland

In recognition of Prof Bradley S. Launikonis outstanding contributions to physiology research and AuPS, we are pleased to award him the honour of presenting the Invited Lecture at the 2023 meeting.

Professor Bradley Launikonis was trained in muscle physiology at La Trobe University, where he obtained his PhD. Follow his PhD, he was awarded a NHMRC CJ Martin Fellowship for his postdoctoral training at Rush University Medical Centre, Chicago, USA. He is currently a Professor and Head of The Muscle Research Laboratory at The University of Queensland. His research team is focused on understanding fundamental properties of Ca2+ handling in skeletal muscle, with emphasis on ryanodine receptors (RyRs) and thermogenesis.

Plenary Lecture

Prof Andy Hill

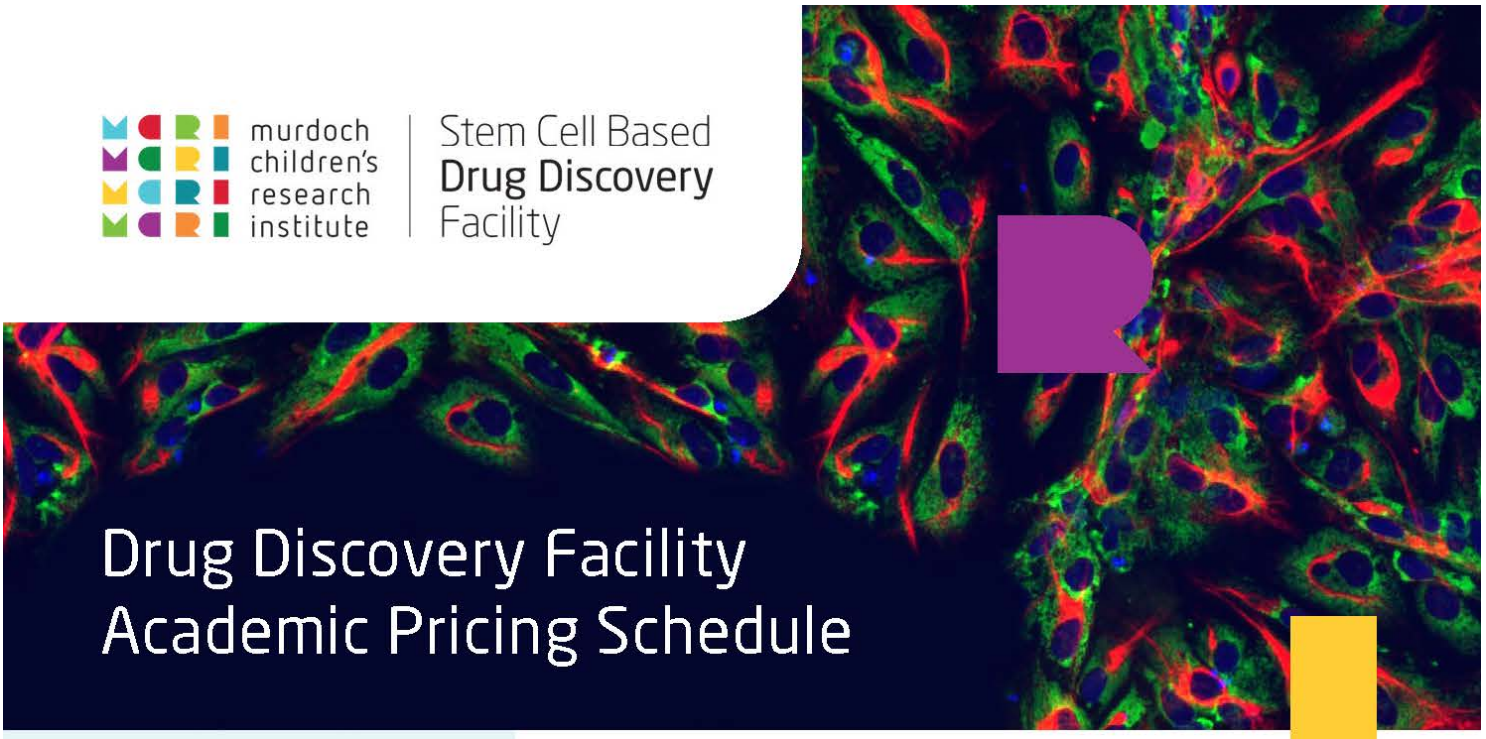
5pm Tuesday

Prof Andy Hill

Victoria University

Prof. Andy Hill is the Deputy Vice-Chancellor Research & Impact at Victoria University. A biochemist and molecular biologist by training, his works focuses on neurodegenerative disorders such as Alzheimer's, Parkinson's and prion diseases. He has a specific interest in understanding the molecular mechanisms by which these proteins exert their neurodegenerative effects and, in the case of prion proteins, gain their infectious properties. More recently, he has established a gold-standard facility in the field of extracellular vesicles at La Trobe University. Andy has published > 200 papers, which have resulted in >33,000 citations of his work and an h-index of 81. He has attracted more than $52 million in research funding as a Chief Investigator over the course of his career.

murdoch children's research institute | Stem Cell Based **Drug Discovery** Facility



Drug Discovery Facility Academic Pricing Schedule

About us

The Stafford Fox Drug Discovery Facility at Murdoch Children's Research Institute offers an automated platform for high throughput/high content drug screening of patient's induced Pluripotent Stem Cell (iPSC) derived tissues.

The Drug Discovery Facility aims to accelerate drug discovery through the use of highly predictive human tissue specific cellular models. The DDF offers a fully integrated automation platform designed for imaging highly relevant complex models.

The platform consists of an automated liquid handling system (Fluent1080, TECAN), an automated rotary incubator (Cytomat5, ThermoFisher) and a high-speed confocal-based imaging system (CV8000, Yokogawa).

Services

Services provided by the Drug Discovery Facility are highly specialised and customised to each screen.

Our services can be accessed in two ways: As a **User-based project** or as an **Assisted project**.

User-based projects rely on the lead researchers to operate the equipment following induction training.

Assisted projects are executed by the DFF staff on behalf of the researchers.

Each project will require one, multiple or all of the following activities:

Image-based phenotypic drug screening

1. 2D and 3D High throughput bright field and fluorescence imaging.
2. 2D and 3D High throughput bright field and fluorescence imaging with drug dispensing.
3. Data processing, QC and analysis.

High throughput liquid handling

1. Automated plate protein coating.
2. Automated plate cell seeding.
3. Automated media changes.
4. Automated drug dispensing.
5. Post-processing sample preparation and immunostaining.

Data processing and analysis

1. Data storage.
2. Image processing and algorithm design.
3. Data report.

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THERAPEUTIC INNOVATION AUSTRALIA

AuPS and ANZSSFR co-badged Symposia

The scientific program includes thirteen symposia sessions:

- Musculoskeletal ageing in females
- New Frontiers in the biology of ageing and sarcopenia
- Molecular transducers of skeletal muscle adaptation to exercise, physical inactivity and ageing
- The Muscle nerve interface, central and peripheral implications for frailty
- Extremes in Physiology: From Bench to Podium
- Mitochondria and Movement: latest insights into mitochondrial adaptations to exercise
- The physiology of inherited myopathies
- Cardiovascular consequences of obesity
- Cardiometabolic diseases: insights from mice, mole-rats and (wo)men
- Cardiac Remodelling in Health, Ageing and Disease
- Emerging research into your mother's role in the aetiology of your disease; molecular mechanisms and novel therapeutics
- The gastro-intestinal tract as the gatekeeper for metabolic allostasis
- The interface of calcium release and muscle function in disease and exercise

Free Communication Sessions

- Brain and integrative neurophysiology
- The heart of the problem
- From drugs to signaling networks
- Energy Metabolism
- Exercise Physiology
- Sex matters in physiology
- Disease models in physiology
- Targeting cancer and cachexia

Physiology Education

A two day hybrid stream, featuring prize lectures, workshops, symposia and free communication sessions showcasing the best educational practice from across Australia.

Poster Session

Over 15 posters will line the Level 7 Greg Craven Centre with presenters from AuPS.

Posters will be available for viewing on Monday between 12:30pm-1:30pm and will be presented on Tuesday between 1-2pm.

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Events for Students and ECRs

Workshop

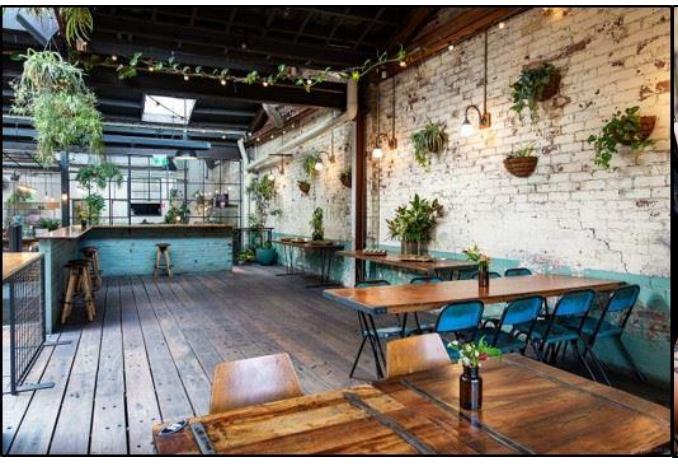
4pm Sunday

The student and ECR workshop is a panel discussion providing insights into career pathways. We have a fantastic line-up of speakers from different backgrounds and career stages including Prof Louise Burke, Prof Andy Jones, Associate Prof Renee Ross and Associate Prof René Koopman. They provide different perspectives around industry-based research, academics working with stakeholders and creating partnerships, and teaching-researcher academics. This will be a fantastic opportunity to hear different and unique career journeys for students and ECRs.

The Mixer

6:30pm Monday

The ECR mixer is always a feature on the conference calendar. This year Stomping Ground Brewery and Beer Hall in Collingwood will serve as host, offering a great chance to relax and have a fun night out with your fellow ECRs.



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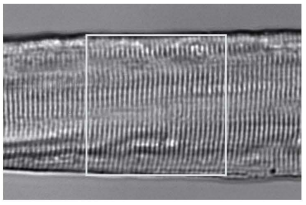
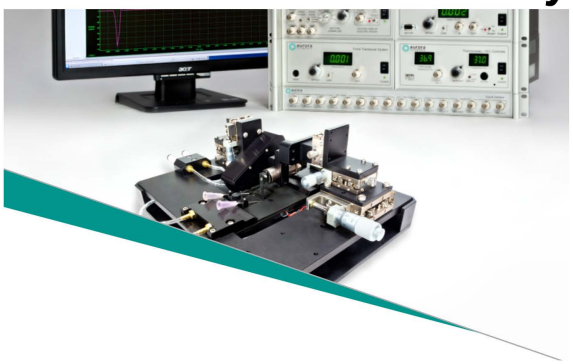
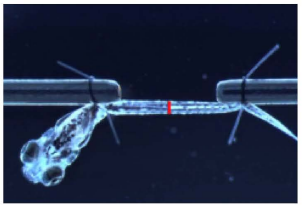
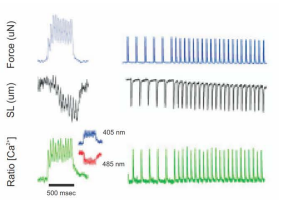


Small Intact Muscle Test System



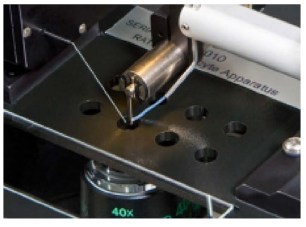
Add FluoroTrack and Sarcomere Length
Powerful Software with Standard Protocols

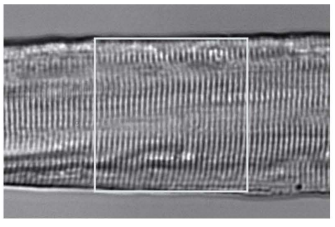




Permeabilized Myocyte Test System

8 Well Plate for Simple pCa Measurement
Integrate with Sarcomere Length
Powerful Software with Standard Protocols





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Physiology Education

Education Symposium

8:30am Tuesday

Virtual Worlds in Biomedical Education

Facilitators: Dr Craig Campbell

Virtual worlds are a growing feature of the educational environment, and this symposium showcases the ways in which they can enhance the educational experience for students in the biomedical sciences. Using immersive virtual environments, students can explore complex concepts and gain hands-on experience in a safe and controlled setting. Students actively participating in the creation of virtual spaces further allows them to apply their knowledge and creativity in a unique and engaging way. Overall, this symposium promises to provide valuable insights into the future of virtual education and its potential to transform the way we learn.

Education Workshop

Tuesday 2pm

Physiology Educator Lab Skills: Cardiovascular (sphygmomanometry) and Neurophysiology (EMG) workshop.

This workshop facilitated by Monash Physiology and ADI will provide educators with hands-on experience and lab skills that can be implemented in their teaching practice.

Free Communication Sessions

Tuesday & Wednesday

Learn from physiology educators from across the country as they share their teaching practice and innovation in three free communication, workshop and networking sessions.

- Gamification, labs and graduate careers
- Student learning and engagement
- Physiology Educators Networking Session

Sunday, 26 November

421.2.03 (Building 421, Level 2)

2pm-5pm **Registration Desk Open**

421.2.03 (Building 421, Level 2)

4pm-5pm **ECR Workshop**

421.2.03 (Building 421, Level 2)

5:15pm- 6:15pm **AuPS Invited Lecture** Chair: Robyn Murphy

Muscle membranes talk

Prof Bradley S. Launikonis - The University of Queensland

ACU Ted Exell Terrace (Building 421, Level 6)

6:30pm - 9pm **Welcome Reception**

The welcome reception is at ACU Ted Exell Terrace, Level 6 and is included in your registration

Monday, 27 November

8am-10am

421.2.03

AuPS/ANZSSFR co-badged day Musculoskeletal ageing in females symposium

Chair: Danielle Hiam

8am	Sex-based difference in skeletal muscle of middle-aged and older adults in response to a period of disuse	Emily J. Arentson-Lantz
8:30am	An 'appetite' for change: preventing falls and fractures in older women	Marc Sim
9am	Factors that challenge ageing muscle and bone in women: an epidemiological perspective	Julie A. Pascoe
9:30am	Mapping the muscle molecular ageing process in females: a lifespan approach	Severine Lamon

421.5.07

Free Communications: Brain and integrative neurophysiology

Chair: Stefan Broer

8am	Targeting Neurofibrosis to Treat Metabolic Disease	Feiyue Shi
8:15am	Unveiling a Hidden Signaling Landscape: Profiling the Secretome of the Medial Basal Hypothalamus in Metabolic Disease	Linda T. Nguyen
8:30am	Mapping the diversity of neuronal populations in the inner ear	Lily J. Pearson
8:45am	Hypothalamic Neurons Expressing the Relaxin Receptor, Rxfp1, Orchestrate Feeding Behaviour and Glycaemic Control for Metabolic Disease Remission in Mice	Sam F. Mohammed
9am	Unraveling a Neuroendocrine Ensemble Orchestrating Metabolic Adaptation in the Hypothalamus	Cait A. Beddows
9:15am	Upgrading Metabolic Disease Treatment by Reversing Glucagon-Like Peptide-1 Resistance in the Brain	Callen C. Goldsmith
9:30am	Unveiling the Role of Reactive Astrogliosis in Neurofibrosis-Driven Metabolic Disease: Implications for Obesity and Type-2 Diabetes	Rui Q. Teo
9:45am	Mitochondrial dynamics and gene expression are impaired in ALS patient derived iPSC-derived lower motor neurons	Leanne Jiang

10am-10:30am

Morning Tea

Greg Craven Centre (Building 421, Level 7)

Monday, 27 November

10:30am - 12:30pm

421.2.03

AuPS/ANZSSFR co-badged day
New Frontiers in the biology of ageing and sarcopenia symposium

Chair: Nir Eynon

10:30am	Exercise rejuvenates the skeletal muscle methylome and transcriptome	Sarah Voisin
11am	Overcoming mechanistic challenges to preserve muscle function in sarcopenia	Gordon S. Lynch
11:30am	Does skeletal muscle stop ageing physiologically?	Avnika A. Ruparelia
12pm	Sarcopenia, the dark side of ageing: the rise of bone metabolism	Itamar Levinger

421.5.07

Free Communications: The Heart of the Problem

Chair: James McNamara + Livia Hool

10:30am	The role of cardiomyocyte cavin-1 in cadiac function and development	Hui Y. Khoo
10:45am	TMEM161B is required for the maintenance of cardiac rhythm	Jessica F. Briffa
11am	TRPM4-Piezo1 signalling axis drives pressure overload-induced cardiac hypertrophy	Yang Guo
11:15am	Understanding the role of Starch Binding Domain containing protein 1 (STBD1) in maintaining cardiac function	Adam Chau
11:30am	Cardiac glycophagy is involved in physiological glycogen handling in the heart post-exercise	James S. L.
11:45am	HUMAN PLURIPOTENT STEM CELL MODELS REVEAL THE MECHANISM OF ALPK3-INDUCED CARDIOMYOPATHY	James McNamara
12pm	Elucidating the role of the cytoskeleton in regulating mitochondrial function in the heart	Teagan Er
12:15pm	Piezo1 is the cardiac mechanosensory that initiates the cardiomyocyte hypertrophic response to pressure overload in adult mice	Ze-Yan Yu

12:30pm-1:30pm

Lunch and Poster Viewing
Greg Craven Centre (Building 421, Level 7)

Monday, 27 November

1:30pm - 3:30pm

421.2.03

AuPS/ANZSSFR co-badged day - Molecular transducers of skeletal muscle adaptation to exercise, physical inactivity and ageing symposium

Chair: Andy Philp

1:30pm	Age-related gene expression changes in skeletal muscle point to potential mechanisms for sarcopenia	Tea Shavlakdze
2pm	Skeletal muscle redox balance in aged conditions	Chrysovalantou E. Xirouchaki
2:30pm	Metabolomic adaptations to prolonged bed rest in energy balance are linked to fuel utilisation and glucose disposal	Isabelle Alldritt
3pm	Acetylation as fundamental regulator of contractile function	Simon Schenk

421.5.07

Free Communications: From drugs to signalling networks

Chair: Natalie Trevaskis

1:30pm	Hypochlorous acid exposure impairs skeletal muscle function and Ca2+ signalling: implications for Duchenne muscular dystrophy	Thomas A. Lea
1:45pm	Redistribution of calcium content in slow-twitch skeletal muscle fibres mediated by SR Ca2+ leak	Cedric R. Lamboley
2pm	Importance of vitamin D for limiting immobilization-induced muscle loss	Memphis Calzoni
2:15pm	Towards the treatment of Duchenne Muscular Dystrophy: Targeting Inflammation with PK007	Sai Yarlagadda
2:30pm	Smooth muscle contractions in the urinary bladder: Alterations between juvenile and adult detrusor and the influences of G protein-coupled receptor stimulation	Charlotte Phelps
2:45pm	Testosterone accelerates differentiation in young but not replicatively aged mouse skeletal muscle cells	Ross Williams
3pm	Detrusor responses to antimuscarinics medications is consistent between juvenile and adult urinary bladders in porcine tissue	Vineesha Veer

3:30pm- 4pm

Afternoon Tea

Greg Craven Centre (Building 421, Level 7)

Monday, 27 November

4pm - 6pm

421.2.03

AuPS/ANZSSFR co-badged day - The Muscle-nerve interface, central and peripheral implications for frailty symposium

Chair: Adam Haag and Peter Noakes

3:45pm	Understanding the NMJ's defects in cancer cachexia	Roberta Sartori
4:20pm	Muscle and neurons in ALS: partners in crime?	Shyuan Ngo
4:45pm	Targeting central feeding circuits to improve outcomes in cancer cachexia	Sarah H. Lockie
5:10pm	Investigating the contribution of neuromuscular signaling and inflammation in neuromuscular diseases: ALS/MND and muscular dystrophy	Peter G. Noakes
5:35pm	From denervation to senescence: exploring the multifaceted signalling in skeletal muscle of geriatric mice	Dan Ham

421.5.07

Free Communications: Energy Metabolism

Chair: Sean Yan

4pm	Phosphoproteomics-directed manipulation reveals SEC22B as an hepatic signaling node governing metabolic actions of glucagon	Yuqin Wu
4:15pm	Interrogating the therapeutic potential of Hexosaminidase A in advanced liver disease	Sihan Lin
4:30pm	Polyamine metabolism regulates muscle stem cell and fibro-adipogenic progenitor proliferation and diferentiation	John H. Nguyen
4:45pm	Hepatic dynamin-related protein 1 silencing affects glucose metabolism independent of sex and body weight	Patricia M. Rusu
5pm	All NASH-HCC models are wrong, but some are more useful than others	Benoit Smeuninx
5:15pm	Targeting hepatic HIBCH for the treatment of non-alcoholic fatty liver disease	Zhili Cheng
5:30pm	Dietary vitamin B12 deficiency reduces white adipose tissue expression of insulin signalling genes in female rats	Elliott S. Neal
5:45pm	Genome-wide CRISPR screen identifies CBX4 as a novel regulator of hepatic lipid metabolism	Li Dong

6:30pm- 10pm

ECR Mixer at Stomping Ground Brewery and Beer Hall
Collingwood 100 Gipps St. 3066

Tuesday, 28 November

8:30am-10:30am

421.2.03

Symposium: Emerging research into your mother's role in the aetiology of your disease; molecular mechanisms and novel therapeutics

Chair: Deanne Hryciw

8:30am	Maternal cannabis use in pregnancy: impacts on fetal/placental development	David Natale
9am	Maternal nutrition can rescue a nephron deficit in growth-restricted offspring	John F. Bertram
9:30am	Exploring the role of human milk lipids in protecting the infant against non-communicable diseases	Alexandra D. George
10am	Imbalances in fatty acid during pregnancy and outcomes for her child's health	Deanne H. Hryciw

421.5.07

Symposium: The physiology of inherited myopathies (with 5 minute introduction from reNEW Melbourne)

Chair: Kevin Watt

8:30am	Facioscapulohumeral dystrophy (FSHD): Molecular mechanisms and therapeutic considerations	Stephen J. Tapscott
9am	Humans as model organisms, time and again rare disease patients inform fundamental biology	Gina Ravenscroft
9:30am	Cardiac trabecular fate is genetically hardwired prior to sprouting morphogenesis	Kelly A. Smith
10am	The importance of patient advocacy in the treatment of congenital heart failure	Adam Piers

Greg Craven Centre (Level 7)

Education Symposium (with 5 minute introduction from ADI)

Chair: Craig Campbell

8:30am	Creating immersive learning environments in higher education	Mohamed Elzein
9am	Virtual Reality in Higher Education	Charles Sevigny
9:30am	Digital teaching in anatomy	Michelle Rank
10am	Utilising the metaverse in biomedical teaching	Christian Moro

10:30am- 11am

Morning Tea
Greg Craven Centre (Building 421, Level 7)

Tuesday, 28 November

11am-1pm

421.2.03

Symposium: Cardiovascular consquences of obesity (with 5 minute introduction from ADI)

Chair: Kate Weeks

11am	HDAC11 inhibition blocks adipocyte lipolysis through reversible myristoylation of HSL	Emma L. Robinson
11:30am	Cardiac adipose: the good, the bad and the unknown	James R. Bell
12pm	Modulating membrane lipids as therapeutics for cardiovascular disease	Yow Keat Tham
12:30pm	DOHaD: Adult Obesity, Heart Disease and Diabetes	Janna Morrison

421.5.07

A Joint Physiological Society of Japan and Australian Physiological Society Symposium: The interface of calcium release and muscle function in disease and exercise

Chair: Robyn Murphy

11am	Dyfunctional muscle Ca2+-thermal signaling in malignant hyperthermia model mice	Toshiko Yamazawa
11:30am	Development of RyR2 inhibitors and verification of their effects in CPVT mouse models	Nagomi Kurebayashi
12pm	Calcium dynamics in a recessive ryanodine receptor myopathy	Daniel P. Singh
12:30pm	Exercise induced fragmentation of the ryanodine receptor: A story of time, muscle fibre type, and fitness for physiological adaptations in skeletal muscle	Barnaby P. Frankish

Greg Craven Centre (Level 7)

Education Free Communications: Gamification, labs and graduate careers

Chair: Julia Choate

11am	Employing Serious Games to Improve Undergraduate Student Engagement	Craig Campbell
11:15am	Crafty teaching or just a game? Educator and student experience of using Minecraft in physiology education	Ben Perry
11:30am	Physiology murder mystery: a two-part workshop to teach undergraduate digestive and metabolic physiology	Michael Leung
11:45am	Utilising LinkedIn for tracking graduate outcomes to advance undergraduate physiology	Christine Lee
12pm	Enhancing Student Engagement in Laboratories: Implementing Pass/Fail Lab Skill Competencies	Chantal Hoppe
12:15pm	Online laboratory experimental simulations foster skills and enhance the student experience of undergraduate Physiology practical laboratory classes	Simone Carron
12:30pm	Can online simulated practical classes replace face-to-face classes in an undergraduate physiology subject?	Angelina Fong
12:45pm	Utilizing a Physiological Simulator for Enhanced Physiology Education	Joseph A.

1pm-2pm

Lunch and Poster Presenting
Greg Craven Centre (Building 421, Level 7)

Tuesday, 28 November

2pm-4pm

421.2.03

Symposium: Cardiometabolic diseases: insights from mice, mole-rats, ischemia and immuno-responsivity

Chair: Kim Mellor

2pm	Co-option of liver glycogen handeling enzymes in the naked mole-rat heart	Jane Reznick
2:30pm	Signalling mechanisms underlying cardiac wound healing	Ronald J. Vagnozzi
3pm	Diabetic heart disease: characterising molecular and cellular impacts of glycogen processing dysregulation	Lea Delbridge
3:30pm	New therapeutic targets for cardiac ischemia	Nathan Palpant

421.5.07

Symposium: The gastro-intestinal tract as the gatekeeper for metabolic allostasis

Chair: Adam Rose and Mark Febbraio

2pm	Dysregulation of amino acid and sphingolipid metabolism in co-morbidities of diabetes	Christian Metallo
2:30pm	Intestinal lymph interactions in metabolic control	Natalie Trevaskis
3pm	Intestinal amino acid transporters as critical gatekeepers of nutrient homeostasis	Stefan Broer
3:30pm	Intestinal ceramide metabolism and metabolic health	Sarah Turpin-Nolan

421.4.04

Free Communications: Muscle Adaptation and Exercise Physiology

Chair: Sophie Broome

2pm	Exercise, but not glucocorticoids, modifies circulating levels of lipocalin-2 and its forms in young males	Carlie Bauer
2:15pm	Repairing muscle without steem cells after exercise	William Roman
2:30pm	Urolithin A Reduces Markers of Inflammation and Muscle Damage, But Does Not Further Improve Endurance Running Performance in Well-Trained Males	Jamie Whitfield
2:45pm	Extracellular vesicles are enriched in activated AMPK following acute exercise	Nimna Perera
3pm	The Ubiquitinomics of Skeletal Muscle Hypertrophy	Craig A. Goodman
3:15pm	Heat Shock Proteins in human single skeletal muscle fibres resist age associated alterations and differentially respond to high-intensity exercise training	Noni Frankenberg
3:30pm	Interrogating the biological roles of dystrophin and utrophin in contraction-mediated adaptations to dystrophic skeletal muscle	Justin P. Hardee
3:45pm	Duality of the FHL1 gene in myopathy and exercise performance	Saveen Giri

Education hands-on lab workshop
with Julia Choate and ADI Instruments 2pm-4pm
Greg Craven Centre Level 7

4pm-4:15pm

Afternoon Tea
Greg Craven Centre (Building 421, Level 7)

4:15pm-4:45pm	Michael Roberts Prize Lecture (421.2.03)
5pm-6pm	AuPS invited speaker (421.2.03)

AuPS Conference Dinner
at the Plaza Ball Room from 6:30pm

Wednesday, 29 November

9am -11am

421.4.04

Symposium: Cardiac remodelling in Health, Ageing and Disease

Chair: Vijay Rajagopal

9am	Crosstalk between Inositol 1,4,5 trisphosphate receptors (InsP3R) and Ryanodine Receptors (RyR) contributes to disrupted Ca2+ handling and arrhythmogenic activity in human heart failure	H. Llewelyn Roderick
9:30am	Phosphoinositide 3-kinase protects against atrial enlargement, fibrosis and thrombi	Julie R. McMullen
10am	An in vitro model that recapitulates the stiff myocardium and hypertrophic cardiomyopathy disease progression	Livia C. Hool
10:30am	Age-specific changes in myocardial molecular expression of human left ventricular tissues from the Sydney Heart Bank	Vijay Rajagopal

421.5.07

Symposium: Extremes in Physiology: From Bench to Podium

Chairs: John Hawley and Nir Eynon

9am	Physiological determinants of extreme endurance exercise performance: lessons from the first sub-2 hour marathon	Andrew M. Jones
9:30am	The molecular athlete: From molecules to medals	John A. Hawley
10am	Survival of the sleepiest: how sleep deprivation impacts athlete physiology and performance	Olivia E. Knowles
10:30am	Freeways and Intersections – Medicine, Sport and Science	Andrew Garnham

Greg Craven Centre (Level 7)

Education Launch of the Core Concepts (Start at 10am)

Chair: Kathy Tangalakis

10am	Unpacking and validating the "physiological adaptation" core concept of physiology	Suzanne Estaphan

11am- 11:15am

Morning Tea

Greg Craven Centre (Building 421, Level 7)

Wednesday, 29 November

11:15am-1:15pm

421.4.04

Free Communications: Sex matters in physiology

Chair: Kate Weeks

11:15am	Investigating sex differences in early systemic and cardiac responses to overnutrition	Adam J. Trewin
11:30am	Fetal growth restriction (FGR) alters cardiac metabolism in a sex- and cause-of-FGR-specific manner in the fetal guinea pig heart	Melanie R. Bertossa
11:45am	Pericardial adiposity increases more rapidly in response to a high fat diet in males vs female rats	Sarah Hayes
12pm	The effect of gestational stress on femoral bone microarchitecture of the dystrophin-deficient mdx mouse	Saba Gharibi
12:15pm	A super complex issue: are there sex-specific changes in mitochondrial respiratory supercomplex assembly following high-intensity interval training in humans?	Dale Taylor
12:30pm	Cardiomyocyte subcellular lipid localisation is associated with severe diastolic dysfunction in female Heart Failure with preserved Ejection Fraction (HFpEF) and diabetic comorbidity	Antonia Raaijmakers
12:45pm	Exercise-responsive gene transcription is differentially affected by an interleukin-6 promotor variant in male and female mice	Lauren E. Watson
1pm	The role of oestrogen in female skeletal muscle ageing: A systematic review	Annabel J. Crichlow

421.5.07

Free Communications: Disease Models in Physiology

Chair: Leonit Kiriaev

11:15am	Serial notexin injury in wild-type mice produces f unctionally normal regenerated skeletal muscle with increased fibre branching that's protected from eccentric injury	Leonit Kiriaev
11:30am	Investigating skeletal muscle dysfunction in a mouse model of critical illness	Amy J. Bongetti
11:45am	Distribution of dysferlin in distinct membrane regions in rat skeletal muscle	Stefan G. Wette
12pm	Mitochondrial Ca2+ handling in mdx mouse	Rhayanna B. Gaglianone
12:15pm	Defective lysosome reformation during autophagy causes skeletal muscle disease	Meagan J. McGrath
12:30pm	Associations Between Muscle-Specific Detrimental Phenotypes in Dysferlin-Deficient Mice and Altered Calcium Handling	Aldo Maizoso-Huesca
12:45pm	Correlating cellular changes with neuromuscular function in Hirschsprung disease	Narges Mahdavian
1pm	Manipulating muscle growth to improve dystrophic pathology in mouse models of Duchenne muscular dystrophy	Wenlan Li

Greg Craven Centre (Level 7)

Education Free Communications: Student Learning and engagement

Chair: Kay Colthorpe

11:15am	Drivers of student engagement and disenagement in a first year human movement studies physiology course	Luli Faber
11:30am	Supporting Introductory Physiology lecture program with interactive online tutorials: impact on students' learning experience and outcomes	Nicole Y. Marden
11:45am	A Deep Dive into FirstYear Bioscience Students' Mindset and Self-Efficacy	Danielle Burgess
12pm	Collaborative peer observation learning circles for ongoing professional development	Sinnayah P.
12:15pm	Exploring teamwork awareness, beliefs and strategies of Undergraduate and Masters of Pharmaceutical Science students	Emilee Molcik
12:30pm	The Impact of Parasocial Interactions on Student Engagement in Blended Learning Environments	Louise Ainscough
12:45pm	Stretching for relevance: connecting the relevance of learning to enhance task authenticity	Judit Kibedi

1:15pm-2:15pm

Lunch + AGM
421.5.07

Wednesday, 29 November

3p-5pm

421.4.04

Free Communications: Targeting cancer and cachexia

Chair: Justin Hardee

3pm	Chronic stress abrogates the beneficial effects of exercise in murine models of breast cancer	Lauren Terry
3:15pm	Post-Developmental Disruption of Muscle Pol1 Exonuclease Activity Induces Mitochondrial Stress and a Cachexia-like Phenotype	Simon T. Bond
3:30pm	TGF-β1 induces metastatic colonisation to skeletal muscle in mouse models of metastatic breast cancer	Alastair A.E. Saunders
3:45pm	Is loss of GFRAL neurons protective in a mouse model of cancer cachexia?	Yunpeng Liu
4pm	Targeting AMPK to improve survival in a colon-26 mouse model of cancer	Emily S. Haber
4:15pm	Regulation of a Novel Splice Variant of Early Growth Response 4 (EGR4-S) by HER+ Signalling and Heat Shock Factor 1 in Breast Cancer	Jeremy M. Drake
4:30pm	Phosphorylation of dystrophin S3059 protects against skeletal muscle wasting in mice	Kristy Swiderski
4:45pm	Dysferlinopathy and myofibre-type specific differences: Further investigation of protein and functional changes in dysferlin-deficient muscles	Erin M. Lloyd

421.5.07

Symposium: Mitochondria and Movement: latest insights into mitochondrial adaptations to exercise

Chair: David Bishop

3pm	Mitochondrial bioenergetics across the movement spectrum	Graham P. Holloway
3:30pm	Using 'omics' to understand mitochondrial adaptations to different types of exercise	David J. Bishop
4pm	Mitochondrial redox regulation in adaptation to exercise	Sophie C. Broome
4:30pm	Regulating the powerhouse – removing mitochondrial bias and avoiding some of the pitfalls in exercise proteomics	Nikeisha J. Caruna

Greg Craven Centre (Level 7)

Physiology Educators Networking Session

Lunch and Posters

Greg Craven Centre (Building 421, Level 7)

Poster 1	Hannah Lalunio	Empagliflozin may positively affect advanced dystrophic muscle
Poster 2	Irene Tsioutsias	LPS-induced skeletal muscle dysfunction is not caused by neutrophil derived reactive oxygen species
Poster 3	Navabeh Zare-kookandeh	Investigating the Effects of Exercise Intensity on Muscle-derived Mediators of Neuroprotection
Poster 4	Aneta Stafanidis	The dichotomy of bariatric surgery: Investigation of the metabolic benefits and adverse behavioral outcomes - insights from an animal model
Poster 5	Zhenhuan Wang	Physiological responses to graded exercise testing and high-intensity interval exercise under normoxia and hypoxia in trained and untrained individuals
Poster 6	Gizel Ruiz	Interrogating skeletal muscle plasticity in response to growth and oxidative stimuli
Poster 7	Wayne X. Du	Unravelling the role of deubiquitinase Ubiquitin-Specific-Protease-15 in skeletal muscle
Poster 8	Yanie Tan	The long non-Coding RNA OIP5-AS1 is sufficient but not necessary for skeletal muscle differentiation in murine models
Poster 9	Jin D. Chung	Intrinsic muscle stem cell dysfunction in Akita mice, a model of type 1 diabetese
Poster 10	Michael S.M. Mah	Contribution of intestinal ceramides to whole-body metabolic dysfunction
Poster 11	Zijing Zhou / Charles D. Cox	MyoD-family inhibitor proteins act as auxiliary subunits of PIEZO channels
Poster 12	Danush Murali	Eccentric contractions, passive stretches and redox modulation increase nuclear translocation of mechanosensitive transcription factor yes-associated protein in fast-twitch skeletal muscle fibres
Poster 13	Yaan-Kit Ng	Affinity purification-mass spectrometry and single fibre proteomics reveals mechanistic insights of C18ORF25
Poster 14	Lily J. Pearson	Mapping the diversity of neuronal populations in the inner ear
Poster 15	Benjamin Strahan	A Hypothalamic circuit regulates immunity in accordance with whole-body energy balance
Poster 16	Melissa E. Reichelt	AAV-targeting of cardiomyocytes to protect hearts from Trastuzumab-induced cardiomyopathy
Poster 17	Olivia JT Smith	Examination of Mitochondrial calcium uniporter complex proteins in the mdx mouse model of Duchenne muscular dystrophy
Poster 18	Ronnie Blazev	Characterising the role of UFMylation in skeletal muscle
Poster 19	Nicholas Giourmas	Therapeutic effect of β-Hydroxy-β-Methylbutyrate supplementation on the mdx mouse phenotype
Poster 20	Shanie Landen	Mechanisms underlying sex differences in Parkinson's disease

ABSTRACTS

Physiological responses to graded exercise testing and high-intensity interval exercise under normoxia and hypoxia in trained and untrained individuals

Zhenhuan Wang[1], Jia Li[1], Muhammed M. Atakan[2], Hiu Tung Tin[1], Jujiao Kuang[1], David J Bishop[1], Li Peng[5], Michael J McKenna [1,5,6], Wentao Lin[6], Xu Yan[1,3,4]

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Introduction: It is well-established that hypoxia (e.g., normobaric or hypobaric conditions) results in a decrease in peak power output (PPO), peak oxygen uptake ($\dot{V}$O2peak), and other physiological parameters. High-intensity interval exercise (HIIE) is a time-efficient approach that offers substantial physical and health benefits. However, the effect of combining HIIE with hypoxia on physiological variables in trained and untrained individuals has yet to be thoroughly investigated, especially when considering a comparative perspective. The aim of this study was to examine potential differences in physiological parameters in untrained and trained individuals in response to graded exercise test (GXT) and HIIE conducted under hypoxic and normoxic conditions.

Methods: Twenty-three male participants volunteered for this study and were divided into two groups based on a $\dot{V}$O2peak threshold of 45 ml/kg/min: untrained group (n=12, $\dot{V}$O2peak = 36.2±5.5 ml/kg/min) and trained group (n=11, $\dot{V}$O2peak = 53.1±8.9 ml/kg/min). All participants completed four graded exercise tests (GXTs): two in normoxia (FiO2 = 0.209) and two in hypoxia (FiO2 = 0.140). Following this, participants engaged in three HIIE sessions, with one-week intervals between each session. These sessions were conducted in random order and included one in hypoxia (HY), one in normoxia matched for absolute intensity with hypoxia (NA), and one in normoxia matched for relative intensity with hypoxia (NR).

Results: During the GXTs, there was a significant decrease in peak heart rate, PPO, lactate threshold (LT), and $\dot{V}$O2peak in both untrained and trained groups under hypoxia compared to normoxia ($p<0.05$). Only a significant main effect of group × condition interaction was observed in $\dot{V}$O2peak ($p=0.013$; partial eta squared ($\eta p2$) =0.26), showing that $\dot{V}$O2peak was the sole physiological variable that exhibited a statistically significant difference during the GXTs between the groups (untrained vs trained: mean difference (Δ) = -5.5 ml/kg/min in hypoxia vs Δ= -11.4 ml/kg/min in hypoxia). In HIIEs, ratings of perceived exertion (RPE) and lactate levels immediately after HIIE in HY and NR were significantly higher than in NA in both trained and untrained groups ($p<0.01$), with no main effect of group or group × condition ($p>0.05$). In the untrained group, there was a significant decrease in oxygen consumption ($\dot{V}$O2) in HY compared to NR ($p<0.001$), while no such change was observed in the trained participants ($p>0.05$). No differences were noted in glucose levels among the HIIE conditions or between the groups ($p>0.05$).

Conclusion: The GXT and HIIE performed under hypoxic conditions seem to have varying effects on physiological variables, especially $\dot{V}$O2, in both untrained and trained individuals.

Is loss of GFRAL neurons protective in a mouse model of cancer cachexia?

Yunpeng Liu[1], Nikita Bajaj[1] & Sarah H. Lockie[1]

[1.] Monash Biomedicine Discovery Institute, Dept of Physiology, Monash University, Clayton, Vic

Background:
Cachexia is a metabolic syndrome caused by chronic illnesses. Approximately 50% of cancer patients experience cachexia, and it contributes to approximately 30% of cancer-related deaths. Cachexia leads to fatigue, weight loss, and reduced food consumption, which, in turn, decreases patients' tolerance to treatments. Currently, there is no known treatment that can completely reverse these symptoms. Growth/differentiation factor-15 (GDF15) is a stress-related cytokine that exhibits relatively high levels in patients with chronic illnesses, including cancer, and is involved in body weight regulation. The receptor for GDF15 is GDNF family receptor α-like (GFRAL), which is expressed only in a small population of neurons in the hindbrain. This suggests potential role for GFRAL-expressing neurons in regulation of metabolic control in cancer cachexia.
Aim:
To investigate how the ablation of GFRAL neurons in the brainstem changes metabolic and appetite regulation a mouse model of cancer cachexia.
Method:
We used mice expressing diphtheria toxin (DT) receptors exclusively in GFRAL-containing neurons using the Cre-Lox system, allowing for the targeted killing of GFRAL neurons with DT via IP injection. We then subjected mice to a model of pancreatic cancer. Briefly, mice lacking GFRAL neurons, and their intact littermates, received an IP injection of pancreatic ductal adenocarcinoma (PDAC) cells that derived from C57BL/6 $KRAS^{G12D}$ $P53^{R172H}$ Pdx-$Cre^{+/+}$ (KPC) mice under light isoflurane anaesthesia, and subsequently developed pancreatic cancer. Food intake was measured continuously using a BioDAQ® Home Cage food intake system. Body composition was measured using echoMRI. Post mortem, we used immunohistochemistry to examine changes in organs and tissues. T-tests and ANOVA were used, as appropriate, to compare food intake, body mass, and tissue changes.
Results:
After 14 days of pancreatic cancer, mice lacking GFRAL neurons had significantly less fat mass loss and body weight loss than intact WT mice. Food intake appeared to decrease all mice with cancer over the 14 days, irrespective of the presence of GFRAL neurons. In the mice lacking GFRAL neurons, there was reduced tumour burden. GFRAL ablated mice had significantly reduced tumour mass compared to WT tumour-bearing mice. Furthermore, 3 out of 8 mice did not develop detectable tumours.
Conclusion:
• The absence of GFRAL neurons can prevent fat mass wasting and body weight loss in a mouse model of pancreatic cancer
• The absence of GFRAL neurons may moderately increase food intake in tumour-bearing mice, but it does not change feeding architecture. This may be partly due to behavioural mimicry of eating when tumour bearing mice are housed alongside healthy mice.
• Effects on body composition may be due to reduced tumour burden, rather than direct effects on whole body metabolism.
• Potentially, tumour development is restrained by ablation of GFRAL neurons, which suggests an unrevealed tumour growth signalling pathway.

Phosphoproteomics-directed manipulation reveals SEC22B as an hepatic signaling node governing metabolic actions of glucagon

Yuqin Wu[1,2], Patricia M. Rusu[1,2], Ashish Foollee[1,2], Andrea Chan[1,2], Susanne Hille[3,4], Jana Hauke[5], Matthew Challis[2], Enzo Huang[2,6], Ralf. B. Schittenhelm[2,6], Luke Formosa[2], Greg C. Smith[7], Jürgen G. Okun[5], Oliver J. Müller[3,4], Adam J. Rose[1,2].

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The peptide hormone glucagon is a fundamental metabolic regulator that is also being considered as a pharmocotherapeutic option for obesity and type 2 diabetes [1-2]. Despite this, we know very little of how glucagon exerts its pleiotropic metabolic actions. Given that the liver is a chief site of action, we conducted time-resolved phosphoproteomics using a perfused rat liver model to reveal glucagon signalling nodes. On pathway analysis of the thousands of phosphosites affected, we identified “vesicle transport” as a dominant signature with the vesicle trafficking protein SEC22 Homolog B (SEC22B) S137 phosphorylation being a top hit. Adeno-associated virus (AAV) mediated hepatocyte-specific loss- and gain-of-function experiments revealed that SEC22B was a key regulator of glucose, lipid and amino acid metabolism, with SEC22B-S137 phosphorylation only controlling distinct aspects such as serum lipid and amino acid metabolism. To explore potential mechanisms, we conducted immuno-precipitation (IP)-proteomics and found most enriched SEC22B binding proteins are involved in vesicle-mediated transport. Glucagon treatment substantially induced more SEC22B binding partners which are involved in lipid and amino acid metabolism pathways, which were absent in the SEC22B-S137A mutant. In summary, here we demonstrate that phosphorylation of SEC22B is a hepatic signalling node modulating the metabolic actions of glucagon, and provide a rich resource for future investigations on the biology of glucagon action.

References

[1] Jastreboff, A. M., Kaplan, L. M., Frías, J. P., Wu, Q., Du, Y., Gurbuz, S., ... & Hartman, M. L. (2023). Triple-Hormone-Receptor Agonist Retatrutide for Obesity - A Phase 2 Trial. *New England Journal of Medicine*, **389**(6), 514-526.

[2] Urva, S., Coskun, T., Loh, M. T., Du, Y., Thomas, M. K., Gurbuz, S., ... & Milicevic, Z. (2022). LY3437943, a novel triple GIP, GLP-1, and glucagon receptor agonist in people with type 2 diabetes: a phase 1b, multicentre, double-blind, placebo-controlled, randomised, multiple-ascending dose trial. *The Lancet*, **400**(10366), 1869-1881.

Distribution of dysferlin in distinct membrane regions in rat skeletal muscle

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Dysferlin is a member of the ferlin family of transmembrane proteins that when absent or dysfunctional results in limb girdle muscular dystrophy 2B (LGMD2B) or Miyoshi myopathy (collectively dysferlinopathies), resulting in muscle atrophy and weakness. Whilst the mechanism that leads to muscle impairment in dysferlinopathies remains unclear, there is evidence to support a role for dysferlin in membrane repair and maintenance of t-systems (1, 2). Several independent studies have shown that dysferlin localises to the surface membrane of muscle (sarcolemma) and the deep invaginations of surface membrane, the t-tubules in mature skeletal muscle when examined using confocal microscopy (3-5). However, the proportion of dysferlin in these distinct membrane regions has not been determined because the signal intensity of proteins attained from confocal microscopy cannot be calibrated and hence accurately quantified. For researchers to better understand how dysferlin dysfunction leads to dysferlinopathy, it is fundamental to determine how much dysferlin is present in the different regions.
To answer this question, extensor digitorum longus (EDL) muscles were dissected from male Sprague-Dawley rats following carbon dioxide asphyxiation in accordance with the La Trobe University Ethics Committee. Individual fibre segments were dissected and mechanically skinned (or "peeled") half-way and both the skinned and intact regions were collected for analysis of dysferlin protein levels by calibrated Western blotting (n = 22 fibres from 6 rats). In a subset of experiments, the sarcolemmal region that forms a "cuff" when the fibre is skinned was excised and collected along with the skinned and intact regions from the same fibre and analysed by calibrated Western blotting (n = 12 fibres from 6 rats). Confocal microscopy analysis of dysferlin localisation was also performed on a subset of mechanically skinned EDL fibre segments (n = 3 rats). Caveolin-3 was used as a positive control for these experiments based on our previous work (6).
We found that ~85% of dysferlin was inside the fibre with no detectable dysferlin in the excised sarcolemma by western blot analysis (n = 7 cuffs). Confocal analysis revealed that dysferlin was located throughout the skinned fibre, possibly at the t-tubular or sarcoplasmic reticulum (SR) membrane. Consistent with previous findings, most (~84%) of the caveolin-3 was inside the fibres, concentrated at the necks of the t-tubules (6). Further analyses are underway to determine if dysferlin is associated with the t-tubules or the SR. These findings indicate that most of the dysferlin protein is not at the sarcolemma. It will be necessary for future studies to focus on understanding the function of this deeper pool of dysferlin in skeletal muscle.

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Signaling Mechanisms Underlying Cardiac Wound Healing

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Tissue repair in the injured heart after myocardial infarction (MI) is a dynamic process which involves the coordinated activity of immune cells and fibroblasts. Proper scar formation following MI is required for adequate healing and maintenance of cardiac function; however excessive scarring leads to pathological fibrosis which can promote cardiac dysfunction, adverse remodeling, and propensity towards heart failure. Pathological inflammation and fibrosis occur in the vast majority of cardiovascular diseases and significantly contribute to heart failure burden; yet therapeutics that directly act to balance the immune-fibrosis axis are lacking. Recent data from our lab and others has suggested one way this balance is achieved in vivo is by selective activity of certain subsets of macrophages, as macrophages can modulate fibroblast phenotype directly as well as modulate the surrounding extracellular matrix (ECM) in the post-MI scar. Specifically, our prior work revealed an unforeseen paradigm where activating a subset of innate immune cells, cardiac tissue-resident macrophages (TRMs) expressing the chemokine receptor CX3CR1 (CX3), improved cardiac wound healing and limited fibrosis after MI in a mouse model. In addition, genetic CX3 deletion in mice rendered the post-MI heart increasingly susceptible to acute rupture and early-onset mortality. We are currently leveraging genetic macrophage tracking and cutting-edge multi-omics, biophysical, and molecular assays of cardiac fibrosis to elucidate how CX3 signaling in TRMs controls infarct scar maturation, inflammation resolution, and functional outcomes post-MI. Our ongoing studies will determine whether $CX3^{+}$ tissue macrophages are a nodal positive regulator of post-MI healing and scarring responses in the infarcted heart, which may provide insight as to how the inflammatory response can be therapeutically balanced to achieve meaningful heart repair following myocardial infarction.

Detrusor responses to antimuscarinics medications is consistent between juvenile and adult urinary bladders in porcine tissue

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Introduction & Objectives: The first-line pharmaceutical therapies for managing overactive bladder (OAB) are antimuscarinics (Moro et al., 2011). Their main mechanism of inhibiting spontaneous contractions in the urinary bladder during the filling phase by blocking the action of acetylcholine in the detrusor, has been identified in juvenile models (Veer et al., 2023). The prevalence of OAB increases with age and it is not clear whether this is due to changes in the urinary bladder tissue structure itself, alterations in lifestyle or other causes (Suskind, 2017). In order to assess the changes to urinary bladder itself, this study aims to find the differences in the ability of commonly prescribed antimuscarinics to inhibit contractions of the detrusor and compare these responses in juvenile and adult porcine tissues.

Methods: Strips of porcine detrusor from the adult or juvenile model were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol concentration-response curves performed in the absence or presence of oxybutynin (1μM), solifenacin (1μM) darifenacin (100nM), tolterodine (1μM), trospium (100nM) and fesoterodine (100nM). Concentrations were chosen to ensure complete concentration-response curves in response to carbachol. pEC50 values for each curve were analysed and estimated affinities calculated. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food.

Results: A right parallel shift was produced from the control in the juvenile detrusor for all antimuscarinics, with estimated affinities calculated for oxybutynin (7.47, n = 10) solifenacin (6.73, n = 8), darifenacin (7.58, n = 11), tolterodine (8.09, n = 8), trospium (8.69, n = 8) and fesoterodine (8.67, n = 8). A right parallel shift was produced from the control in the adult detrusor for all antimuscarinics, with estimated affinities calculated for oxybutynin (7.44, n = 9) solifenacin (6.63, n = 8), darifenacin (7.95, n = 9), tolterodine (7.93, n = 8), trospium (9.30, n = 9) and fesoterodine (8.54, n = 8). Comparisons of estimated affinities for each antimuscarinic between juvenile and adult tissues revealed no differences in each tissue's functional response to the six antimuscarinics ($p > 0.05$).

Conclusions: Although preliminary, with this study ongoing, there appears to be no significant differences between detrusor functional responses to antimuscarinics of differently aged porcine samples. Further supporting that these medications can assist in the treatment of OAB and lower urinary tract symptoms in the detrusor layer. Differences in compliance may be due to lifestyle or behavioral changes with age rather than alterations in the tissues ability to respond to the prescribed medication themselves.

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Investigating sex differences in early systemic and cardiac responses to overnutrition

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Cardiovascular disease is the leading cause of death in people with diabetes. Women with diabetes are particularly vulnerable, with a 4-fold increased risk of developing heart failure compared with a 2-fold increase in risk for men. Diastolic dysfunction is a key feature of the diabetic heart which contributes to the development of heart failure. Despite clear clinical and epidemiological evidence, there are no gender-specific diagnostic or treatment strategies for diabetic heart disease. Improved understanding of the mechanisms contributing to differential gender vulnerability in diabetes is needed for the development of targeted therapies. The aim of this study was to characterise the emergence of diastolic dysfunction in male and female rats during the development of type 2 diabetes induced by overnutrition. We compared systemic and cardiac parameters in rats exposed to two different diets: a high fat (HF) diet that has been widely used in rodent overnutrition studies, and a high fat and high sugar diet (HFHS) that more closely resembles the Western diet.

Adult male and female Wistar rats were randomly assigned to the HF diet (Specialty Feeds SF04-001), HFHS diet (SF00-219) or a control diet (CON, AIN93G) at n=6 per sex and diet group. Following a 1 week transition feeding period, rats consumed the diet *ad libitum* for 4 weeks. At endpoint (12 weeks of age), a fasted glucose tolerance test (GTT) was performed (bolus i.p. injection of glucose 1.5 g/kg) with blood glucose measured at pre-specified timepoints for 2 hours to calculate area under the curve (AUC). Flow and tissue Doppler echocardiography was performed on anaesthetised rats (inhalation of 1.5% isoflurane) to assess diastolic function. Rats were then euthanised with pentobarbital (20 mg/kg i.p.) and heart tissue weighed and collected for transcriptional and proteomic profiling.

In male rats at endpoint, body weight was ~10% higher in the HF and HFHS groups compared to CON (P<0.05). Fasted blood glucose levels and AUC from the GTT tended to be higher with HF or HFHS feeding but were not statistically significant. Heart weight normalised to tibia length in males was also unaffected by HF or HFHS feeding. In female rats, both the HF and HFHS diets increased body weight (~13% and ~8% increase respectively vs CON, P<0.05; no significant difference between HF and HFHS). Fasted blood glucose levels were elevated (~15% and ~13% increase in HF and HFHS vs CON; P<0.05) and the GTT revealed impaired glucose tolerance in both the female HF and HFHS groups (~50% and ~30% increases in AUC vs CON, P<0.05; no significant difference between HF and HFHS). Female rats in the HF group also showed signs of cardiac hypertrophy (~13% increase in heart weight normalised to tibia length vs CON, P<0.05). Echocardiography revealed diastolic dysfunction in both sexes with both diets. E/e' ratio (a marker of stiffness) was significantly increased in both male and female rats fed a HF or HFHS diet (P<0.05), while mitral valve deceleration times were significantly reduced (P<0.05). In males, there were no differences in E/e' between the HF and HFHS groups. In females, the HFHS diet appeared to have a greater effect on E/e' due to reduced movement of the mitral valve annulus (e' wave), but this requires further investigation.

In summary, a relatively short period of overnutrition was sufficient to induce diastolic dysfunction in both male and female rats. Glucose intolerance was more pronounced in female rats than in male rats, and female rats fed a HF diet developed cardiac hypertrophy, suggesting that females are more susceptible to the pathological consequences of overnutrition. Ongoing work is investigating transcriptional and proteomic changes that occur in the male and female heart with HF and HFHS feeding.

Intestinal lymph interactions in metabolic control

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Background: Excess visceral adipose tissue (VAT) increases the risk of insulin resistance (IR) by releasing pathogenic factors that impair insulin signalling. VAT encases mesenteric lymphatic vessels through which lymph is transported from the intestine. Recently, we found that mesenteric lymphatic vessels become highly branched and leak lymph to VAT, promoting IR in obese high fat diet (HFD) fed mice. The mesenteric lymphatic dysfunction was regulated by prostaglandin E2 synthesised by cyclooxygenase-2 (COX-2), which stimulated the release of the pro-lymphangiogenic factor VEGF-C from macrophages in VAT. Mesenteric lymphatic dysfunction and IR were reversed with a lymph-directed prodrug of COX2 inhibitor celecoxib. Our next aim is to determine the role of gut-microbiota products in regulating obesity-associated mesenteric lymphatic vessel dysfunction that drives insulin resistance.

Methods: Mice were fed a control diet (CFD) or HFD, or HFD for 15 weeks, then switched to CFD, given an oral antibiotic (AB) to deplete microbiota and housed with bedding from CFD or HFD mice for up to 21 weeks (Fig 1a). Some HFD fed mice were treated with CCR2 inhibitor INCB334432 or TLR/Myd88 inhibitor T6167923 to determine the role of macrophages and TLR/Myd88 activation. Lymphatic and VAT structure was profiled with immunofluorescence analysis, mesenteric lymph leakage was quantified with Evans blue lymphangiography and obesity and glucose intolerance were tracked from body weight and composition, oral glucose and insulin tolerance tests. Lymph was collected via cannulation of the mesenteric lymph duct under 2% isoflurane anaesthesia to measure flow and composition which was analysed by lipidomics, ELISAs and flow cytometry.

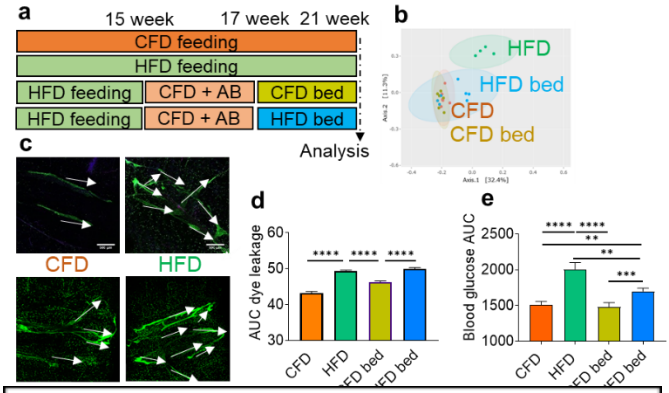

Fig 1: Study timeline. **b)** PcoA plot of faecal microbiota profiles in each group, **c)** Mesenteric lymphatic branching images (lyve-1, green) **d** Lymph leakage (blue dye AUC), **e** Blood glucose AUC over 90 mins after oral glucose.

Results: Antiobitic treatment of mice with HFD induced obesity followed by housing with bedding from CFD or HFD fed mice led to re/population with lean or obese conditioned gut microbiota, respectively (Fig 1b). Repopulation with lean but not obese conditioned gut microbiota was accompanied by a reduction in mesenteric lymphatic branching and leakiness (Fig 1c-d), and improved oral glucose tolerance (Fig 1e) with little effect on total body weight.

Conclusion: Altogether this data supports that gut microbiota are critical drivers of mesenteric lymphatic dysfunction, and consequently glucose intolerance, in mice with HFD-induced obesity. Modulation of the gut microbiota or their effects with diets, biotics or other medical approaches therefore represents a potential treatment strategy to combat obesity-associated mesenteric lymphatic dysfunction and associated insulin resistance.

AAV-targeting of cardiomyocytes to protect hearts from Trastuzumab-induced cardiomyopathy

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Amplification of HER2 occurs in 20% of breast cancers. HER2 (known as ErbB2 in mouse) belongs to the Epidermal Growth Factor Receptor (EGFR) family that is responsible for cell growth and survival. While HER2 does not bind ligands, it forms heterodimers with HER1 (also known as EGFR) in the presence of its ligand EGF, and HER4 following stimulation with Neuregulin. In HER2-positive breast cancer where HER2 expression is high, HER2 forms homodimers leading to uncontrolled activation of downstream signalling and cell proliferation. Current HER2-positive breast cancer treatment involves chemotherapy with drugs called anthracyclines, such as Doxorubicin, and an antibody targeting HER2 called Trastuzumab. This combination is effective in killing HER2-positive breast tumours, however, Trastuzumab alone or in combination with anthracyclines leads to severe cardiotoxicity and heart failure in 2.6-11% of cases, making it a dose-limiting side effect. We hypothesise that cardiac toxicity can be mitigated by targeting cardiomyocytes exclusively to express a mutated (but functional) version of HER2 that is not recognised by trastuzumab. We designed 3 HER2 variants wherein the trastuzumab binding domain was mutated to disrupt binding. These HER2 mutants were transfected into HEK293T cells, and we confirmed that all 3 successfully localised to the cell membrane, and did not bind trastuzumab. We have used bioluminescence resonance energy transfer to examine the capacity of all 3 mutants to activate recruitment of Grb2 to the HER2 as part of HER1/HER2 and HER4/HER2 heterodimers; Western blotting also confirming mutants were capable of stimulating ERK and Akt phosphorylation. Future studies will evaluate the efficacy of using AAVs to instruct cardiomyocytes to express these HER2 mutants in a murine model of breast cancer. We predict that this new therapy would not only mitigate cardiac toxicity, but would also permit higher doses of trastuzumab to be used to treat breast cancer.

Elucidating the role of the cytoskeleton in regulating mitochondrial function in the heart

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Background: Cardiomyocytes are high energy consuming cells that must be able to meet energy demands on a beat-to-beat basis. It is well recognised that calcium is required to maintain ATP production by mitochondria. Previously we identified that the L-type calcium channel can alter mitochondrial membrane potential in a calcium-independent manner. This occurs via a structural-functional link between the L-type calcium channel and the mitochondria that involves the cytoskeleton. Impairment of the cytoskeletal network is associated with dysregulation of mitochondrial function and the development of pathological states. The auxiliary beta subunit of the channel is tethered to the large subsarcolemmal protein AHNAK that also binds F-actin, and uncoupling the site attenuates the increase in mitochondrial membrane potential. However the downstream mechanism by which the cytoskeletal network modulates mitochondrial function in the heart remains elusive.
Methods: Cardiomyocytes were isolated from wild-type mice in accordance with approval from The University of Western Australia Animal Ethics Committee. Mitochondrial membrane potential was measured as changes in JC-1 fluorescence following activation of the L-type calcium channel by the dihydropyridine agonist BayK(-)(10μM). All experiments were performed under calcium-free conditions in the presence or absence of 5μM Latrunculin A to depolymerise F-actin.
Results: Application of BayK(-) caused a 22.31 ± 3.39% increase in JC-1 signal (n=12) that was attenuated when cells were preincubated with Latrunculin A (6.31 ± 1.451% increase, n=12; $p<0.05$). Application of 20μM oligomycin (inhibitor of ATP synthase) alone resulted in a similar increase in JC-1 signal to BayK(-) (27.52 ± 4.81% n=7) and preincubation of cells with Latrunculin A did not alter the response (25.66± 5.17% increase, n=5, p=NS vs oligomycin alone). However application of a peptide that binds the voltage dependent anion channel (VDAC) mimicked the increase in JC-1 by BayK(-) (30.83 ± 1.81%, n=7, p=NS vs BayK(-) alone).
Conclusions: Our data suggest that F-actin does not mediate alterations in mitochondrial membrane potential via the ATP synthase but may be facilitating the increase in mitochondrial membrane potential via the voltage dependent anion channel.

Targeting Neurofibrosis to Treat Metabolic Disease

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Type-2 diabetes has emerged as an escalating global health challenge, witnessing a staggering 129.7% surge over the past two decades. Notably, 86% of diabetic patients are concomitantly diagnosed with obesity. Within the arcuate nucleus (ARC) of the hypothalamus, a critical centre for metabolic regulation, the pathophysiological process known as neurofibrosis unfolds during the progression of obesity. The resultant augmented and remodelled extracellular matrix within the ARC significantly contributes to cellular insulin resistance, precipitating systemic metabolic dysfunction. Remarkably, there are currently no therapeutics targeting neurofibrosis.

To address this unmet need, we have pioneered several "first-in-class" small molecule inhibitors, termed PZX and its analogues. We have identified that PZX delivered intranasally administration to obese mice over a ten-day period can cross the blood-brain barrier and ameliorate neurofibrosis within the ARC. This therapeutic attenuation of neurofibrosis resulted in profound weight loss, reduced food intake, heightened energy expenditure, diminished adiposity, and enhanced glycaemic control in diet-induced obesity. Further mechanistic insights revealed that the protective actions of PZX on metabolism were mediated through insulin receptor signalling in agouti-related peptide (AgRP) neurons within the ARC. Employing CRISPR-Cas9-mediated genetic manipulation, we selectively ablated insulin receptors in ARC AgRP neurons, which attenuated the effects of PZX on weight loss and glycaemic improvement. The efficacy of this novel therapeutic mechanism was subsequently validated using both structural and functional analogues of PZX, further reinforcing its potential for enhancing energy homeostasis and glycaemic control in vivo.

We further explored if a combination therapy of neurofibrosis inhibitor could potentiate the actions of the GLP-1 receptor agonist, Liraglutide, for the treatment of metabolic diseases. To explore this, we co-administered liraglutide with or without PZX in diet-induced obese mice. This intervention not only attenuated neurofibrosis but also facilitated liraglutide's access to the ARC parenchyma. Consequently, the augmented ARC GLP-1 receptor signalling translated into substantial improvements, with mice receiving the combination therapy displaying significant and sustained weight loss, reduced adiposity, lower food intake, enhanced carbohydrate utilisation, and improved glycaemic regulation. Collectively, our findings unveil a novel promising class of therapeutics that holds the potential to alleviate brain insulin and GLP-1 resistance, ushering in new prospects for the treatment of metabolic diseases.

Phosphorylation of dystrophin S3059 protects against skeletal muscle wasting in mice

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The dystrophin-glycoprotein complex (DGC) is a multi-protein structure required to maintain integrity of the muscle fibre membrane, transmit force, and maintain muscle homeostasis. Membrane localisation of dystrophin is perturbed in muscles wasting in conditions associated with chronic inflammation (Swiderski *et al*., 2014). Through proteomics and mutagenesis studies, we identified novel phosphorylated residues within endogenous dystrophin, and that phosphorylation at serine 3059 (S3059) enhanced interaction between dystrophin and β-dystroglycan, another key DGC protein (Swiderski *et al.*, 2014). *In vitro* testing revealed that mimicking S3059 phosphorylation by mutating to glutamate (S3059E) attenuated C2C12 myotube wasting in the presence of colon-26 (C-26) cancer cells (Swiderski *et al*., 2021). In the present study, we investigated the role of S3059 phosphorylation on skeletal muscle *in vivo,* testing the hypothesis that dystrophin S3059 phosphorylation is fundamental to the aetiology of muscle wasting.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (NHMRC). BALB/c mice with a systemic mutation of dystrophin S3059 to either alanine (DmdS3059A) or glutamate (DmdS3059E) were generated with CRISPR/Cas9. Male BALB/c wild type (WT), DmdS3059A, and DmdS3059E mice were either anaesthetised (sodium pentobarbitol, 60 mg/kg, *i.p.*) for *in situ* muscle function testing at 3, 6, or 12 months of age, or anaesthetised (oxygen-isoflurane, induction - 3-4% at 0.8 L/min, maintenance - 2-3% at 0.8 L/min, inhalation) and given a subcutaneous injection of phosphate buffered saline (PBS; control) or C-26 cancer cells passaged once (mild cachexia) or twice (severe cachexia) into the right flank at 10-12 weeks of age, then anaesthetised deeply with sodium pentobarbitol (60 mg/kg, *i.p.*) for muscle function testing 12-34 days later. The quadriceps, extensor digitorum longus (EDL), gastrocnemius, and tibialis anterior (TA) muscles were excised for biochemical and histological analyses, and mice were killed by cardiac excision while anaesthetised deeply.

Muscle mass relative to body mass tended to reduce with age from 3 to 12 months in DmdS3059E mice, which was not observed in DmdS3059A mice, but neither mutation increased susceptibility to contraction-mediated muscle damage like that which occurs with an absence of dystrophin. With mild cancer cachexia, DmdS3059A mice exhibited greater loss of muscle mass than WT and DmdS3059E mice, which was maintained in severe cancer cachexia. While WT and DmdS3059E mice exhibited cachexia at endpoint in the severe model, DmdS3059E mice lived longer than WT mice (24 days vs. 17.5 days; $P < 0.01$). The survival rate of DmdS3059A with severe cachexia was not different from WT (18 days vs. 17.5 days; $P = 0.3946$). Gene expression analyses confirmed attenuated expression of atrophy markers, MuRF1, Atrogin-1, and LC3B, in EDL muscles from DmdS3059E mice with advancing cachexia relative to both WT and DmdS3059A mice.

These findings demonstrate that the S3059A mutation accelerates, and the S3059E mutation slows cachexia progression in C-26 tumour-bearing mice, which may be attributed to altered regulation of the atrophy program. Determining the mechanisms underlying post-translational modification of S3059 could identify novel targets to restore DGC interactions to protect skeletal muscles in wasting conditions.

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Calcium dynamics in a recessive ryanodine receptor myopathy

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Ryanodine receptor 1 (RyR1) are the Ca2+ release channels of skeletal muscle fibres that regulate muscle contraction. In the event of mutated RyRs the outcome for an individual can range from no obvious effect on muscle strength through to very severe and life-shortening myopathy. In most cases the inheritance of recessive RYR1 mutations from both parents leads to the most severe forms of the myopathy (compound heterozygous). There are no effective treatments for RYR1-related myopathies (RYR1 RM). A major obstacle has been the lack of knowledge surrounding the pathomechanisms of recessive RYR1 RM. We know that the function of mutated RyR1 channels are altered in RYR1 RMs compared to normal RyR1s. What we don't know is how changes in the local movements of Ca2+ due to the change in RyR1 activity affect how the immediate cellular environment responds to these changes. This is likely to be critical to the health status of the muscle. In this project we used an uncharacterised compound heterozygous mouse model of recessive RYR1 RM that directly mimics the severe progressive early onset muscle weakness observed in children.
Three groups of mice were examined: wild type, single recessive mutation and compound heterozygous (two recessive mutations). The mice were euthanised via cervical dislocation then the extensor digitorum longus (EDL) muscles were rapidly removed and pinned to Sylgard set in a Petri dish containing paraffin oil. Single fibres were isolated and mechanically skinned then assayed using several approaches: (i) confocal imaging of a Ca2+ sensitive dye trapped in the t-system to act as a nano-domain Ca2+ sensor of RyR activity; (ii) a fibre-lysis method where released Ca2+ from membraneous compartments is quantified from the force response generated, to determine the calcium content of the SR or mitochondria; and (iii) a heat sensitive fluorescent dye (ER thermo-yellow) as a direct measure of the contribution of dysfunctional RyR1 activity towards heat generation and consequent ATP turn-over within individual muscle fibres. Our results showed that a greater number of mutations caused a greater RyR Ca^{2+} leak and re-distribution of Ca^{2+} with a compromise to contractile performance (compound heterozygous > single mutation > wild type). Our heat measures showed a significant increase in heat generation and consequent ATP usage in the compound heterozygous model versus the wild type. The severe RyR1 dysfunction observed in the compound heterozygous mice suggests that several downstream effects are triggered to cause considerable muscle weakness. The substantial metabolic demands (energy expenditure) required to re-distribute Ca^{2+} and the resulting oxidative stress from the mitochondria likely play an important role in the pathogenesis of the disease. Together, we were able to highlight several compromised compartments within the RyR1 myopathy model which leads to a cascade of events and eventual muscle weakness.

An ‘appetite’ for change: preventing falls & fractures in older women

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One in three individuals over 65 years will experience a fall annually. In Australia, falls are the leading cause of hospitalised injuries and injury deaths among older individuals. In this age group, 50% of falls result in fracture, incurring an average in-patient hospital stay of 9 days. Of importance, older women also present with a much higher risk of falls and fractures compared to their male counterparts; warranting separate investigation. In addition to the trauma and injury associated with the fall and/or fracture itself, patients often have prolonged impaired mobility remaining functionally dependent upon others.

Exercise has emerged as one key factor in lowering fall and fracture risk, likely through its ability to improve muscle function and prevent chronic disease. But exercise is only one piece of the puzzle: nutrition is also significant. From a dietary perspective, attention has primarily been on the benefits of supplementing protein, vitamin D and calcium. However, the importance of other aspects of diet for musculoskeletal health to prevent falls and fracture remain largely unknown.

Recently however, diets rich in vegetable-derived nitrate have been linked to better muscle strength of the upper and lower-limbs, as well as physical function in adults across the lifespan. Notably, both green leafy and cruciferous vegetables are rich sources of vitamin K that is essential for the carboxylation of the bone-derived protein osteocalcin, which has been implicated in the material properties of bone. Our recent data also indicates dietary vitamin K1 may play an important role for fracture and falls prevention in older women. In summary, this talk will cover the importance of diet for musculoskeletal health, whilst also examining novel strategies to screen for nutritional deficiencies (e.g. muscle biomarkers, DXA whole-body imaging) to identify and manage individuals with high risk of falls and fractures.

Age-related gene expression changes in skeletal muscle point to potential mechanisms for sarcopenia

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To understand the changes in gene expression that occur as a result of age in skeletal muscles, we generated musti-tissue, multi-time point age-related gene expression signatures in mice and rats, as these are the most commonly used species in preclinical research and intervention testing. Male and female C57Bl6J mice and male Sprague Dawley rats were used at ages 6, 9, 12, 18, 21, 24 and 27-months. The lower limb muscles (gastrocnemius, tibialis anterior and soleus) and the diaphragm were analyzed. We identified age-related genes that change in one direction throughout the lifespan of the animal, either early in life (early logistic changes), at mid-age (mid-logistic), late in life (late-logistic), or linearly. We next compared age-related transcriptomic profiles between mice and rats. Overall, more age-related genes were identified in rat skeletal muscles compared with mice, among which the gradual, linearly altered genes predominate. This was consistent with the finding that rat muscle undergoes more robust-age-related declines in mass. In both species, pathways associated with inflammation and apoptosis gradually increased with age. Pathways linked with extracellular matrix remodeling were universally down-regulated. Interestingly, reduced expression in pathways linked to energy generation and mitochondrial function was seen in rats, but not mice. This extensive, side-by-side transcriptomic profiling shows that skeletal muscle aging in rats is impacted by aging via a more gradual process during their life span, compared with mice. Wether rats are a better model for human sarcopenia needs to be further established.

We used the rat model of sarcopenia to test potential pharmacological intervention by targeting the mTORC1 (mammalian target of rapamycin complex 1) pathway. Paradoxically, mTORC1, a well-established positive modulator of muscle mass, is hyperactivated in sarcopenic muscle. Furthermore, partial inhibition of the mTORC1 pathway counteracted sarcopenia, as determined by observing an increase in muscle mass and fiber type cross-sectional area in select muscle groups. Additionally, several genes related to senescence were downregulated and gene expression indicators of neuromuscular junction denervation were diminished using a low dose of a "rapalog" (a pharmacological agent related to rapamycin). Therefore, partial mTORC1 inhibition may delay the progression of sarcopenia by directly and indirectly modulating multiple age-associated pathways, implicating mTORC1 as a therapeutic target to treat sarcopenia.

Pericardial adiposity increases more rapidly in response to a high fat diet in males vs female rats

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Atrial fibrillation is the most common sustained arrhythmia, characterised by irregular and chaotic beating of the atria. The onset of arrhythmias can be caused by regional areas of slowed conduction across cardiomyocytes, introducing 're-entrant' electrical pathways. Obesity is a key risk factor for the development of atrial fibrillation. Notably, pericardial adipose accumulation has been independently associated with conduction abnormalities that underlie vulnerability to atrial fibrillation. Increased cardiac adiposity is associated with infiltration of adipose into the epicardium as well as increased fibrosis. We recently identified a novel intercellular communication axis between pericardial adipose and cardiomyocytes, whereby pericardial adipose can release paracrine factors that stimulate fibrosis and slow cardiomyocyte properties. To date, the field has primarily investigated the relationship between cardiac adiposity and atrial fibrillation at single timepoints, typically when the pathologies are at an advanced stage. A longitudinal evaluation of the sequence of events by which cardiac adiposity contributes to the development of the atrial substrate for atrial fibrillation has yet to be reported. Additionally, very little is known about the relative influence of cardiac adiposity in males and females. We now aim to address both knowledge gaps and map the development of cardiac adiposity and atrial electrophysiological properties in males and females.
8-week-old male and female Wistar fats were fed a high-fat (SF04-001, 43% fat) or a control diet (AIN93G, 16% fat) for 4 weeks (n=6 rats per group). Rats were euthanised (pentobarbital, 20mg/kg dose) and atrial and pericardial adipose tissues collected and weighed.
At 4 weeks of feeding, both male and female rats exhibited a significant increase in body weight compared with control diet fed rats. No diet or sex differences in total heart or atrial weights were observed. Interestingly, pericardial adipose tissue weight (both total and relative to body weight) increased in high fat diet fed male rats, but not females.
These data indicate that males are especially vulnerable to a rapid growth of pericardial adipose tissue in response to a relatively short period of high fat feeding. Further studies will extend the longitudinal assessment of adiposity accumulation in high fat diet fed rats, with a focus on understanding how paracrine influence on atrial conduction properties change as adiposity develops.

Duality of the *FHL1* gene in myopathy and exercise performance
Samuel

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FHL1 mutations cause a range of muscle disorders including Reducing Body Myopathy (RBM) and Emery-Dreifuss Muscular Dystrophy (EDMD) ("FHL1 myopathies", OMIMs: 300163, 300695, 300696, 300717, 300718, 300280, 310300). Despite over a decade since their discovery, the disease mechanism and cause of their marked clinical heterogeneity remains enigmatic. These can be rapidly progressive infant/childhood onset disorders, fatal within 5 years due to cardiorespiratory failure (RBM). We provided the first insight, revealing mutant FHL1 protein accumulates as aggregates linked to disease onset and progression. Others report milder disorders including EDMD, where mutations result in FHL1 protein loss from muscle. In a new breakthrough, we generated unique FHL1 mouse models that recapitulate this clinical spectrum, for analysis of disease mechanism(s) and pre-clinical evaluation of treatments. We also uncovered a new role for FHL1 in maintaining muscle mitochondrial function. FHL1 loss reduces the mitochondrial network expanse and its energy-generating function thereby decreasing muscle strength, exercise tolerance and enhancing fatigue. Intriguingly *FHL1* is also an exercise-responsive muscle gene. Increasing wild type FHL1 in muscle (by as little as 4-fold in transgenic mice), was sufficient to stimulate the effects of endurance exercise training including marked expansion of the mitochondrial network that enhances energy production and exercise performance. Advanced volumetric electron microscopy revealed marked changes to mitochondrial structure in muscle when wild type FHL1 levels are elevated compared to when it is lost. Therefore, *FHL1* is a gene at the nexus of muscle disease and athleticism, with a new opportunity to develop mitochondria-targeted therapies for FHL1 myopathies.

Towards the Treatment of Duchenne Muscular Dystrophy: Targeting Inflammation with PK007

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Duchenne muscular dystrophy (DMD) is a devastating genetic disorder characterized by progressive muscle weakness and degeneration, primarily due to the absence of dystrophin protein. The acute and chronic phases of DMD are marked by heightened inflammation, with prostaglandin D2 (PGD2) playing a significant role as one of the drivers of this process.

Three-week-old male C75BL/10 ScSn-mdx (n = 12) and C75BL/10 ScSn (strain control; n = 12) mice were used in this study, sourced from the Animal Resources Centre (ARC) Perth, WA Australia. Mice were randomly allocated into two groups (n = 6) and were housed in individually ventilated cages (6 pups per cage). These mice were treated in a double-blind manner with vehicle (0.5% methyl cellulose, 0.1% Tween80, and MilliQ water), or HPGDS inhibitor (PK007: 10 mg/kg/day in 0.5% methylcellulose, 0.1% Tween80, and MilliQ water) via oral gavage daily. We conducted a 10-day treatment regimen using PK007, a novel hematopoietic prostaglandin D synthase (HPGDS) inhibitor, in a well-established DMD mouse model (mdx ScSn10 mice). Our primary aim was to evaluate its impact on disease progression, with a specific focus on muscle strength and inflammation. We assessed skeletal muscles, diaphragm, heart, and tongue, along with pro-inflammatory protein/enzyme levels and the potential genes regulating the pro-inflammatory pathway for inflammation.

Grip strength significantly increased in the PK007-treated groups during both the acute and chronic phases of the disease. Moreover, PGD2 levels were markedly decreased in both acute and chronic phases in the PK007-treated group, indicating a reduction in pro-inflammatory activity. Our histological analysis of muscle tissues revealed intriguing findings, including reduced muscle necrosis and a lower percentage of regenerating muscle fibres in the PK007-treated group. Notably, we found TIMP1 and ARG1 expression levels to be increased with PK007 treatment, suggesting a potential modulation of pro-inflammatory pathways as part of the drug's mechanisms.

Our findings suggest that PK007 holds promise as a potential intervention for DMD by effectively reducing inflammation, necrosis, and muscle damage. In conclusion, our study highlights the potential value of targeting the arachidonic acid (AA) pathway to decrease inflammation, thus slowing the progression of the disease, and providing therapeutic relief in muscle strength. However, further investigations are needed to elucidate the drug's mechanisms of action, potential off-target effects, and the regulation of the Arachidonic Acid pathway when PGD2 levels are downregulated.

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The effect of gestational stress on femoral bone microarchitecture of the dystrophin-deficient *mdx* mouse

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Duchenne muscular dystrophy (DMD) is a fatal neuromuscular disease caused by the loss of dystrophin. Loss of dystrophin increases bone fragility both in patients and in the *mdx* mouse model of DMD, which is thought to be driven via skeletal muscle weakness. Here, we examined whether gestational stress, which detrimentally affects numerous physiological phenotypes in offspring, further exacerbates the DMD bone phenotype. C57BL/10-*mdx* heterozygous female mice in the last week of gestation were subjected to stress by either a 30 s scruff-restraint, twice/day or a 30 min tube-restraint twice/day or were left unstressed (n = 16 - 22 per group). Femora from male wildtype and *mdx* offspring from each stress paradigm were collected and trabecular and cortical microarchitecture were analysed at 6, 12 and 24 weeks of age by micro-computed tomography (n = 6 - 12/group/genotype). Independent of stress paradigm, cortical area, mean polar moment of inertia, and femoral length were lower in *mdx* mice at 6 weeks of age relative to wildtype mice ($p < 0.001$). At 6 and 12 weeks of age, *mdx* mice also exhibited lower trabecular bone volume, trabecular thickness, and trabecular number relative to wildtype mice ($p \leq 0.005$). At 24 weeks of age, *mdx* mice displayed lower cortical and trabecular thickness ($p \leq 0.022$), and greater femur length relative to wildtype mice ($p < 0.001$). Since our analysis of stress response demonstrated no statistically significant differences in the impact of scruff-restraint and tube-restraint paradigms, we compared bone structure between "non-stressed" and "stressed" offspring, which included both paradigms. At 6 and 12 weeks of age, gestational stress had no impact on the bone microarchitecture of wildtype or *mdx* mice. However, at 24 weeks of age, in both genotypes, gestational stress was associated with greater marrow area and endocortical perimeter in the diaphysis and greater femur length in the stressed group relative to the non-stressed group ($p \leq 0.045$). These data confirm that dystrophin-deficiency compromises bone microarchitecture in mice and suggests that gestational stress does not affect the bone microarchitecture of wildtype and dystrophin-deficient mice during developmental stages. However, it does indicate that, in older wildtype and dystrophin-deficient mice, gestational stress leads to endocortical expansion and femoral growth.

Hepatic dynamin-related protein 1 silencing affects glucose metabolism independent of sex and body weight.

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Dynamin-related protein 1 (DRP1) is a key protein in mitochondrial and peroxisomal fission and has been shown to affect systemic metabolism. However, whether these changes are dependent on developmental effects of loss-of-function or on changes in body weight are currently unknown. Here we confirm prior studies that germline loss of hepatocyte DRP1 (using Alb-CRE) reduces body weight and fat gain and improves glucose homeostasis on an obesogenic high-fat diet. Hyperinsulinaemic-euglycaemic clamp studies revealed a selectively reduced endogenous glucose production rate, explaining the improved glucose metabolism in the liver DRP1 KO mice. In female mice, the liver DRP1 knockout mice gained equal body and fat weight as controls but had improved glucose metabolism. Similarly, when DRP1 was silenced in hepatocytes of adult obese male mice, improvements in glucose metabolism were seen independent from any difference in body weight. Lastly, male germline hepatic DRP1 KO mice placed on an obesogenic diet for just a few days showed improvements in glucose metabolism before measurable differences in body weight. Taken together, these data suggest that there is an intrinsic effect of hepatic DRP1 silencing on systemic glucose metabolism via reduced hepatic glucose production that is independent from developmental effects or body weight.

Testosterone accelerates differentiation in young but not replicatively aged mouse skeletal muscle cells

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Ageing is accompanied by a progressive loss of skeletal muscle mass, imposing physical and financial burdens on individuals and healthcare systems. In severe cases of muscle wasting, testosterone replacement therapy is often prescribed to limit the loss of muscle mass. However, little is known about the causal effects of testosterone in aged skeletal muscle cells. Prior research in mouse skeletal muscle cells has established the use of replicative ageing through multiple population doublings as an efficient and reproducible model of atrophic phenotypes, with population doubled cells exhibiting an impaired myogenic potential (Sharples et al. 2012). This study aimed to utilise this model to investigate the effects of repeated doses of testosterone in young and replicatively aged mouse skeletal muscle cells.
Young and replicatively aged immortalised C2C12 mouse skeletal muscle cells were induced to differentiate in the presence of a single 100nM dose of testosterone (TSIN), repeated 100nM doses of testosterone (TREP) or a 100nM vehicle control dose of DMSO (CON) for 10 days. Myotubes were live imaged throughout the course of differentiation and the number and diameter of myotubes measured. After 7 days of differentiation a subset of myotubes were immunohistochemically stained to visualise myosin heavy chain and nuclei.
Repeat testosterone administration induced significantly greater increases in the number (TREP 44.8 ± 6.9% vs CON 25.5 ± 6.5%; P = 0.002) and diameter of myotubes (TREP 14.9 ± 1% vs CON 8.5 ± 1%; P = 0.018) between timepoints compared to the control treatment in young cells. These effects persisted until day 5 of differentiation, at which point the diameter of TREP myotubes plateaued. By 10-days differentiation, myotubes had decreased in diameter across all 3 conditions, with a significantly greater decrement in diameter occurring in the TREP myotubes compared to the CON myotubes (CON 4.9 ± 0.4µm vs TREP 4.6 ± 0.3µm; P = 0.025). None of the above effects were observed in the replicatively aged myotubes. These results suggest that testosterone treatment may accelerate the differentiation in young but not replactively aged skeletal muscle cells. Conversely, prior research has demonstrated testosterone's ability to partially restore the myogenic potential of cells having undergone fewer population doublings than those used in this study (Deane et al. 2013; Hughes et al. 2016). Therefore, these results suggest the development of an anabolic resistance in cells subjected to significant numbers of population doublings, reminiscent of the effects of ageing in skeletal muscle in-vivo.

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Mitochondrial Ca^{2+} handling in mdx mouse

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The skeletal muscle is pivotal for conferring and regulating movement, posture, and generating body heat. Within the muscle fibers, the sarcoplasmic reticulum-mitochondria interaction is essential to the regulation of Ca^{2+} and ATP production for the maintenance of these processes. The ryanodine receptor 1 (RyR1) is located on the sarcoplasmic reticulum (SR) and releases Ca^{2+} from SR into the cytosol to regulate the muscle contraction. At rest, the RyR1 leaks Ca^{2+} which must be continuously resequestered by the SR Ca^{2+} pump, in a process requiring ATP hydrolysis. The leak can increase due to mutations, to cellular environment changes, with lifestyle or disease onset. The SR is the primary regulator of Ca^{2+} release- and re-uptake. However, the mitochondria also take up Ca^{2+} depending upon the activity of the SR. Mitochondrial Ca^{2+} is important for regulating ATP resynthesis to support ongoing muscle activity and the Ca^{2+} reuptake by SERCA. Additionally, the plasma membrane Ca^{2+} ATPase is responsible for extruding Ca^{2+} from cytosol back to t-tubule lumen to maintain the large Ca^{2+} gradient across the transverse tubular membrane. In Duchenne Muscular dystrophy (DMD), leaky RyRs cause elevated intracellular Ca^{2+} and potentially increase oxidative stress due to increases in mitochondrial Ca^{2+} content. Here, we are interested in understanding whether mitochondria have raised mitochondrial free Ca^{2+}, the mitochondrial Ca^{2+} handling under multiple Ca^{2+} transients and the RyR leaky state. Using skinned fibers from extensor digitorum longus (EDL) muscle from DMD murine model (mdx) we determined the mitochondrial free [Ca^{2+}] using the FCCP mito spike. With this maneuver we were able to determine the mitochondrial free [Ca^{2+}], which is 242 nM and 432 nM for WT and mdx respectively. Additionally, WT and mdx fibers were exposed to caffeine to evaluate the mitochondrial Ca^{2+} uptake under multiple Ca^{2+} transients. Results evidenced an increase in free mitochondrial Ca^{2+} baseline of 39 nM in WT and 247 nM in mdx, which represents an increase of 19.6% and 57.4% for WT and mdx respectively. We further analyzed the PMCA efficiency to evaluate the Ca^{2+} extrusion process. Preliminary results indicate that the PMCA steady state at 135 nM Ca^{2+} in WT is 0.68 mM Ca^{2+} in absence of tetracaine, while the mdx presented a steady state of 2 mM Ca^{2+} under the same conditions. In the presence of the RyR inhibitor tetracaine, the WT steady state at 135 nM Ca^{2+} is 0.62 mM Ca^{2+} while the mdx is 1.16 mM Ca^{2+} under the same conditions. Altogether, initial results evidence that the RyR leak induces the PMCA overload and the free mitochondrial Ca^{2+} accumulation which may induce to oxidative stress, reduced mitochondrial oxygen consumption and apoptosis.

Age-specific changes in myocardial molecular expression of human left ventricular tissues from the Sydney Heart Bank

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Age is a significant independent risk factor for heart disease, but the molecular underpinnings of this risk factor is unresolved. Using a unique collection of human donor heart tissue samples spanning the human lifespan from the Sydney Heart Bank, we conducted multiomics measurements and bioinformatics analyses of how the molecular profile of the human left ventricle changes with age. We inferred functional consequences of key molecular changes to the heartbeat using biophysics-based computational models of cardiac subcellular processes. In older hearts, we observed a downregulation of proteins involved in calcium signalling and of the contractile apparatus itself. In addition, we found a potential counteractive upregulation of central carbon generation of fuel, upregulation of glycolysis and increases in long-chain fatty acids. This is the first molecular data set of normal human cardiac ageing, which have relevant implications for the development of age-related heart disease.

Cardiomyocyte subcellular lipid localisation is associated with severe diastolic dysfunction in female Heart Failure with preserved Ejection Fraction (HFpEF) and diabetic comorbidity.

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Background: Heart failure with preserved ejection fraction (HFpEF) is now the dominant form of heart failure diagnosis. It is characterised by diastolic dysfunction and ventricular stiffness, and often associated with diabetes, hypertension and obesity comorbidities. Women are especially vulnerable – female HFpEF diagnoses are 2-fold greater relative to males and are more likely to confer premature mortality. Despite clinical observations, very few studies have investigated mechanisms of HFpEF with diabetic comorbidity and the sex specificity of this interaction has not been explored. The aim of this study was to evaluate functional and molecular mechanisms associated with sex differences in a diabetic comorbidity model of HFpEF.

Methods: Diabetes was induced in 30-week male and female rodents with genetic hypertrophy (Hypertrophic Heart Rat, HHR) by streptozotocin (STZ; 55 or 25mg/kg) treatment. At 34-weeks echocardiography was performed under light anaesthesia (inhalation of isoflurane at 1.5%) prior to post-mortem tissue recovery. Data dependent acquisition proteomics was performed to characterize male and female diabetic HFpEF myocardial proteins. Left ventricular sections were stained with Oil red O to determine intracellular lipid content and picrosirius red to examine fibrosis. Furthermore, cardiac lipid droplet accumulation and co-localisation with sarcomeres was histologically evaluated.

Results: HFpEF females with a diabetic comorbidity demonstrated premature mortality and exacerbated diastolic dysfunction (70% increase in E/e', $p<0.05$ compared to males). This was not due to a modification in the extracellular matrix as the extent of fibrosis was unchanged. Lipid droplet quantification indicated a significant increase in cardiac lipid accumulation in diabetic HFpEF females (125%, $p<0.05$) and a strong positive correlation between cardiomyocyte lipid accumulation and diastolic dysfunction was identified ($r=0.72$, $p<0.05$). This apparent link between intracellular lipids and diastolic dysfunction was associated with a downregulation of lipid transport and processing proteins in diabetic female hearts. Additional histological analyses revealed co-localisation of cardiac lipid droplets with sarcomeric proteins in the diabetic female heart and a significant correlation between sarcomeric protein levels and diastolic dysfunction ($r=0.84$, $p<0.05$) was observed.

Conclusion: The strong relation between cardiomyocyte lipid droplet density, sarcomeric localisation and diastolic dysfunction suggests that cardiac lipid accumulation is involved in exacerbating diastolic dysfunction in female diabetic HFpEF. This provides new insight into potential pathways for new therapeutic interventions in HFpEF.

Smooth muscle contractions in the urinary bladder: Alterations between juvenile and adult detrusor and the influences of G protein-coupled receptor stimulation

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Introduction: The prevalence of bladder contractile disorders increases with older age, and the mechanisms within the urinary bladder tissue that are altered remain unknown. There is the potential for dysfunction to be due to receptor changes within the different layers of the urinary bladder (Phelps et al., 2023). Of particular interest is the detrusor smooth muscle which provides the main contractile force during voiding, and as such, variations in this muscle could present in cases of overactive or underactive bladder (Moro et al., 2021). **Aim**: This study will identify if there are alterations to G protein-coupled receptor (GPCR) activation pathways for urinary bladder detrusor contractions between juvenile and adult tissues. **Methods**: Porcine urinary bladders from Large White-Landrace-Duroc cross-bred pigs were used as the tissue in this study. Juvenile samples were taken from prepubescent pigs (6 months old, 80kg live weight) and adult samples from sow pigs (2-3 years old, 200kg live weight). Strips of detrusor smooth muscle were dissected and mounted in functional organ baths containing Krebs-Henseleit bicarbonate solution at 37°C and gassed with carbogen (95% O_2, 5% CO_2). Tissue contractions (grams) were recorded before and after the addition of a single dose of GPCR agonist in the absence and presence of selective inhibitors of extracellular Ca^{2+} influx, intracellular Ca^{2+} release, or Rho kinase. An unpaired Student's two-tailed *t*-test was used to compare responses between juvenile and adult detrusor smooth muscle. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. **Results**: Urinary bladder detrusor smooth muscle from both juvenile and adult animals contracted in response to a single dose application of muscarinic receptor agonist carbachol (1μM), histamine (100μM), 5-HT (100μM), neurokinin-A (300nM), prostaglandin-E2 (PGE2, 10μM), and angiotensin-II (ATII, 100nM). Younger tissue was more sensitive to stimulation with histamine, whereas adult tissue was more sensitive to stimulation by 5-HT, PGE2, and ATII. Inhibition of extracellular Ca^{2+} entry into the tissue or blocking the Rho kinase pathway impacted all contractions, with no difference between juvenile and adult detrusor. Impairment of intracellular Ca^{2+} release inhibited contractile responses to PGE2 in the adult detrusor, but not in the juvenile detrusor. **Conclusions**: The age-related variations in responses to agonist stimulation may provide insights into potential systems that could contribute to dysfunction in urinary bladder contraction.

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Hypochlorous acid has a greater effect on fast-twitch compared to slow-twitch skeletal muscle in wild type and dystrophic mice

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Duchenne muscular dystrophy (DMD) is a fatal X-linked genetic disease which results in severe wasting of skeletal muscle. The mechanisms of DMD are largely unclear but seem to involve chronic inflammation, oxidative stress, and dysregulation of Ca2+ signalling. These mechanisms of DMD are further complicated as fast-twitch fibres are preferentially affected compared to slow-twitch fibres. The mechanisms of this differential effect are largely unclear; however, one hypothesis is a differential susceptibility to the reactive oxygen species (ROS), hypochlorous acid (HOCl). HOCl is a highly potent ROS produced by neutrophils (Terrill et al., 2020), which can affect skeletal muscle function and has been proposed as a unifying link between inflammation, ROS, and altered Ca2+ homeostasis in DMD (Lea et al., 2022). These previous studies have been limited to fast-twitch muscle and it is unknown if these effects can also be exerted in slow-twitch muscle. The aim of this study was to determine the effects of HOCl on contractile function in the primarily slow-twitch whole soleus muscle, along with determining any specific differential effects of HOCl on slow (type I) or fast-twitch (type II) chemically skinned fibres (FSC). Wildtype (C57) and dystrophic (mdx) mice were anaesthetised via an intraperitoneal injection of sodium pentobarbitone (40 mg/kg body weight). Soleus and extensor digitorum longus (EDL) muscles were isolated and mounted in an in vitro muscle test system (1200A; Aurora Scientific, Canada) containing HEPES free Ringers' solution, bubbled with carbogen at 25 °C and selectively exposed to 200 μM HOCl while undergoing a series of isometric contractions. For FSC experimentation, single muscle fibres were isolated from soleus muscle and attached to a force transducer (model: BAM4C, SI Heidelberg) and chemically skinned in Triton X-100 leaving only the contractile filaments intact. FSC were subsequently classified as type I or type II via a Sr2+ sensitivity assay and subsequently exposed to 50 μM HOCl (5 minutes), before again being maximally contracted. In whole muscle experimentation, HOCl significantly decreased maximal force in both EDL (C57: 24 % decrease, mdx: 49 % decrease) and soleus muscles (C57: 14 % decrease, mdx: 13 % decrease) from C57 and mdx mice (p's < 0.05, two-way ANOVA). HOCl exposure caused a greater decrease in maximal force in whole EDL muscle compared to whole soleus muscle (p's < 0.05, two-way ANOVA). In FSC experimentation, HOCl caused a significant reduction in maximal Ca2+ -activated force for both type I and type II fibres isolated from C57 (21 % in type I and 39 % in type II) and mdx mice (34 % in type I and 36 % in type II) (p's < 0.05, paired t-test). The effect of HOCl on maximal Ca2+ activated force was greater in type II fibres in C57 ($p < 0.05$, unpaired t-test) but not mdx. Additionally, HOCl had a greater effect on the time taken by fibres to reach maximal Ca2+ activated force in type II fibres of the soleus muscles from both C57 (80 seconds in type I and 162 seconds in type II) and mdx mice (95 seconds in type I and 110 seconds in type II) , compared to type I fibres (p's < 0.05, unpaired t-test). Our results indicate that HOCl has more severe effects in whole fast-twitch EDL muscle compared to the slowtwitch soleus muscle. This differential effect may be at least in part explained by differential effects on contractile filaments from type I and type II fibres. These results are important for the mechanisms of DMD as they indicate that differential susceptibility to HOCl may potentially in part explain why fast-twitch fibres are preferentially affected compared to slow-twitch fibres. References Lea, T., Pinniger, G., Arthur, P., & Bakker, A. (2022). The Effects of Hypochlorous Acid on Skeletal Muscle Function: Impications for the Pathophysiology of Duchenne Muscular Dystrophy University of Western Australia]. University of Western Australia Terrill, J. R., Al-Mshhdani, B. A., Duong, M. N., Wingate, C. D., Abbas, Z., Baustista, A. P., Bettis, A. K., Balog-Alvarez, C. J., Kornegay, J. N., & Nghiem, P. P. (2020). Oxidative Damage to Urinary Proteins from the Grmd Dog and Mdx Mouse as Biomarkers of Dystropathology in Duchenne Muscular Dystrophy. PloS one, 15, e0240317.

Factors that challenge ageing muscle and bone in women: an epidemiologic perspective

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Introduction: During normal progression through adulthood, declines in skeletal muscle and bone occur somewhat synchronically in response to shared age-related challenges. These might include inflammation, ectopic fat deposits, endocrine imbalances, mood disorders, disuse and unhealthy lifestyles. Examples of challenges are presented, focusing on skeletal muscle decline in women, and drawing on epidemiological data from the Geelong Osteoporosis Study (GOS).
Methods: At baseline (1993-7), 1494 women (ages 20-94y) were recruited from electoral rolls for the Barwon Statistical Division (participation 77%) and re-assessed at 2-yearly intervals. Coincident with the 10-y follow-up, the cohort was supplemented with 246 women ages 20-29y from the 2005 electoral roll. The combined cohort has been assessed at 15y and 25y. Participants have serial measures including anthropometry, dual energy x-ray absorptiometry, tests of muscle function and mental health; diet, lifestyle, exposure to drugs and diseases, and sociodemographic factors are captured by questionnaire. Blood has been collected at 3-timepoints during follow-up.
Results/conclusions: Lean mass peaks in the third age-decade and thereafter declines; age alone explains an estimated 11.9% of the variance in total lean mass and 15.5% in appendicular lean mass (ALM). In 2019, revised criteria for sarcopenia by the European Working Group on Sarcopenia in Older People (EWGSOP2) recommended using the GOS cut-point for low ALM. Criteria for diagnosing sarcopenia lack consensus and different thresholds for poor muscle function with or without consideration of low muscle mass are used. We found that sarcopenia prevalence according to EWGSOP2 identified fewer cases than EWGSOP1, with estimates using the National Institutes of Health between the two.
GOS data are contributing to efforts for identifying and optimising diagnostic cut-points according to their ability to discriminate poor physical function, including consideration for psychological well-being. We report that women with depression have poorer performance in tests of muscle strength and physical performance. While there are biological pathways for a mood-muscle link, consequences of depressive symptoms might also contribute to poorer measures of muscle function, which rely on participant effort. Sarcopenia and obesity were also independently associated with anxiety. We suggest that psychological wellbeing be considered when assessing muscle function as a diagnostic indicator for sarcopenia.
Bedrest and immobility associated with critical illness, in tandem with inflammation and endocrine dysfunction, are associated with rapid musculoskeletal deterioration. We measured changes in body composition in mechanically-ventilated intensive care patients between discharge and one-year follow-up and observed accelerated increases in bone resorption and bone loss compared to GOS controls; further, patients recovered some of the lean mass lost while critically ill, but did not reach the level of GOS peers.
GOS reports that very few early-elderly women undertake resistance‑training despite having the capacity to be physically active. We also investigated age-related patterns of absolute and relative lean mass in association with non-specific physical activity levels described as sedentary, active and very-active. A physical activity dose-dependent increase was observed in mean ALM/BMI across age-groups and the very-active group had a mean adjusted ALM/h^2 that was significantly greater than the sedentary group.
GOS data were used to derive an Australian Recommended Food Score (ARFS), a Dietary Inflammatory Index and *a posteriori* dietary patterns. Over 5y, a higher ARFS score, an anti-inflammatory diet and a traditional dietary pattern all independently predicted higher ALM/h^2, suggesting that a less inflammatory diet and a diet comprising a wide variety of plant and animal foods may be beneficial for preserving muscle mass in women

Muscle and neurons in ALS: partners in crime?

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Amyotrophic lateral sclerosis (ALS) has historically been viewed almost exclusively as a neuron-centric disease where the progressive death of upper and lower motor neurons leads to paralysis and death. Muscle changes are generally thought of as being a consequence of motor neuron loss and neuromuscular denervation. However, multiple studies in ALS indicate that skeletal muscle dysfunction might actively contribute to muscle weakness, as well as to the final demise of neuromuscular junctions and motor neurons.

In this talk, I will discuss metabolic perturbations in muscle that have allowed us to gain a better appreciation of its contribution to disease in ALS. I will touch on our pre-clinical studies in mouse models of ALS, which were key in facilitating the expediting of a therapeutic candidate into clinical trials. Finally, I will touch on some recent work on induced pluripotent stem cell derived motor neurons, where we are aiming to determine whether aberrations in mitochondrial dynamics might lead to neurometabolic abnormalities that increase the susceptibility of motor neurons to death in ALS.

Maternal cannabis use in pregnancy: impacts on fetal/placental development

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Background: Cannabis use is significantly increasing amongst pregnant women. Cannabidiol (CBD), a constituent of cannabis, is often perceived as "natural" and "safe." In-utero cannabis exposure is associated with a myriad of negative health outcomes, including fetal growth restriction (FGR). The placenta functions to supply oxygen and nutrients to the fetus; alterations in placental development can lead to FGR yet the impacts of in-utero CBD exposure on the placenta are unknown. Based on our previous work exposing pregnant rats to the cannabinoid delta-9-tetrahydrocannabinol, we hypothesized that in-utero CBD exposure impacts the structure of and gene expression in, the placenta.

Methodology: Placentas from vehicle and CBD (3 mg/kg) exposed rat pregnancies were collected. Placental structure and gene expression were assessed through immunohistochemistry (IHC) and analyzed via unpaired t-tests. Human placental pericytes (hPlPeri) were cultured with vehicle (0.2 % ethanol) or CBD (2 μM) and were placed in hypoxia or normoxia. Cell confluency was measured and analyzed by unpaired t-tests. Supernatant was collected, Ang1 and VEGF-a were assessed by ELISA and analyzed via unpaired t-tests.

Results: CBD exposed placentas displayed a decreased fetal blood space perimeter to area ratio ($p<0.05$) as well as decreased GLUT1 and GR expression, and increased GLUT3 expression ($p<0.05$). MCT4 expression was decreased in CBD exposed placentas ($p<0.05$), indicating a negative impact on the syncytiotrophoblast II layer. CBD-exposed hPlPeri in normoxia displayed a decreased cell confluency, decreased secretion of Ang1, and an increased secretion of VEGFa ($p<0.05$). CBD-exposed hPlPeri placed in hypoxia displayed a decreased confluency compared to VEH in hypoxia, decreased Ang1 secretion, and decreased VEGFa secretion ($p<0.05$).

Conclusion: Our results show that in-utero exposure to CBD impacts expression of nutrient transporters and the fetal vasculature in the placenta. Further, in-vitro analysis showed that CBD alters angiogenic signaling in exposed hPlPeri.

Correlating cellular changes with neuromuscular function in Hirschsprung disease

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Introduction: Hirschsprung disease (HD) is a life-threatening neuropathy caused by the absence of enteric neurons (aganglionosis) in the distal regions of the colon. HD is treated by the surgical removal or bypass of the aganglionic bowel. Although lifesaving, over 30-50% of patients suffer from postsurgical symptoms including chronic constipation, enterocolitis, and faecal incontinence. This suggests that factors beyond the enteric neurons are impacted in HD, or that the remaining ganglionated bowel may have additional anomalies. We hypothesise that non-neuronal cells with established roles in motility may be impacted in HD. This study aims to determine the distribution and function of cells involved in motility in HD compared to the healthy colon and to correlate this to neuromuscular function.
Methods: Colon samples were collected from 13 HD and 8 control patients (HREC: 38262). Neuromuscular function was assessed by measuring smooth muscle contractions in segments spanning the length of the resected bowel. The contractile effects of electrical and pharmacological stimulation of neurons and smooth muscle were assessed. Spontaneous activity and responses to smooth muscle relaxants were also observed. Immunofluorescence imaging was used to detect and quantify innervation density and distribution of interstitial cells of Cajal (ICC) and PDGFRα+ cells (PC) throughout the external muscle.
Results: There were marked differences in smooth muscle contractions, cell distributions, and innervation along the resected HD colon and with healthy control bowel. Contractions in response to stimuli were larger and more variable in the ganglionated HD bowel compared to the control bowel ($P<0.05$, n=8-13). Neuromuscular function varied along different regions of the resected HD colon (n=13). In 10 of 13 HD patients examined, contractions generated in response to stimulation were larger in the most proximal segment compared to the most distal aganglionic bowel. In 11 of 13 patients, the greatest responses to stimulation occurred in segments from the central regions of the resected colon where ganglia are present but not fully formed. There was a significant reduction in innervation and PC densities between ganglionated HD bowel and healthy controls ($P<0.05$, n=7-8). Neurons, ICC, and PC were variably distributed throughout different regions of the HD colon ($P<0.05$, n=7-8). Our results reflect the heterogeneity of the disease and the differences between the normal and ganglionic HD colon may explain why some patients experience postsurgical issues including chronic constipation.
Conclusions: This study provides novel insight into HD pathophysiology. The differences in cell distribution and associated function between ganglionated HD bowel and healthy control bowel may explain the postsurgical symptoms HD patients experience.

Investigating the Effects of Exercise Intensity on Muscle-derived Mediators of Neuroprotection

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Background: Aerobic exercise training is an effective intervention to protect against age-related brain atrophy and cognitive decline. Growing evidence suggests that these benefits are, at least partly, mediated by 'myokines' released by skeletal muscle during contraction. However, the most effective 'dose' of exercise training to promote beneficial changes in these pathways is currently unknown. Specifically, most of the evidence to date is from studies that have used moderate-intensity continuous training (MICT). Research investigating the potential merit of high-intensity interval training (HIIT) is scarce, despite its well-established health benefits in various aspects of cardiovascular health, metabolic function, and physical fitness. The aim of this study was to investigate the effects of HIIT vs MICT on myokine pathways and outcomes associated with neuroprotection in both human and animal models.

Methods: *Human study* - 32 cognitively normal, middle-aged (45 to 65 y- 3 male, 29 female) adults performed one of two 12-week, work–matched, aerobic exercise training interventions (randomised allocation): HIIT (4 to 7 x 4 min, 90% of peak power) or MICT (36 to 48 min, 60% peak power). Resting blood and muscle samples were collected before and after the training intervention. *Animal Study* - 37 middle-aged (52 to 54 weeks) male C57BL-6 mice were randomly allocated into one of three 6-week interventions: HIIT (4 x 4 min, 90% of max speed), MICT (24 min, 60% of max speed), or passive control (CON). Mice were sacrificed ~72 h after training for the collection of blood, muscle, and brain tissue. *Analyses* - Tissue from both human and animal studies was used to investigate the role of exercise training intensity on myokine pathways (e.g., fibronectin type III domain-containing protein 5, FNDC5), and outcomes (e.g., adult hippocampal neurogenesis), associated with neuroprotection.

Results: *Human study* - Both MICT and HIIT interventions elicited increases in neuroprotective myokines (Cathepsin B, Vascular Endothelial Growth Factor (VEGF)) and upstream markers (Silent Information Regulator 1) in human skeletal muscle. The MICT group exhibited a significant increase in the pERK/tERK ratio. Protein levels of brain-derived neurotrophic factor (BDNF) and FNDC5 in skeletal muscle remained unchanged in both exercise groups. Serum levels of Kynurenine metabolites, and the kynurenine/tryptophan ratio, were increased in both groups (HIIT, P=0.025, MICT, P=0.06). Notably, Kynurenic acid (KA), a neuroprotective factor within the kynurenine pathway, significantly increased only in the HIIT group.
Animal study - We observed decreased brain kynurenine protein levels in both the MICT and HIIT groups. Adult hippocampal neurogenesis demonstrated a trend toward an increase in BrdU-positive cells in both the MICT and HIIT groups. However, these changes did not reach statistical significance.

Discussion and Conclusion: Elevated Cathepsin B and VEGF levels in response to both MICT and HIIT support the role of skeletal muscle in the release of neuroprotective mediators. The pERK/tERK ratio increased in MICT, but not HIIT, suggesting that exercise intensity is a regulator of the ERK pathway following exercise. The elevated kynurenine/tryptophan ratio in human serum represents a pronounced shift in tryptophan metabolism toward the kynurenine pathway. Higher KA levels in the HIIT group highlight the potential neuroprotective effects of high-intensity exercise. In addition, it has been suggested that lower brain kynurenine levels are associated with reduced quinolinic acid (QA) accumulation, a neurotoxic metabolite. This is consistent with our findings in mouse brain, where a shift in tryptophan metabolism away from the kynurenine pathway (i.e., towards serotonin metabolism) may contribute to exercise-induced neuroprotection. Finally, the lack of change in mice adult hippocampal neurogenesis after 6 weeks of exercise, despite an upregulation in myokine pathways, may be explained by the short duration of training (i.e., 6 weeks). Future research investigating the role of longer training interventions on brain markers of neuroprotection is warranted.

In summary, these results highlight the importance of exercise intensity in modulating neuroprotective mechanisms. Specifically, our findings suggest that high-intensity exercise training may augment myokine pathways and outcomes associated with neuroprotection compared with moderate-intensity exercise training. Further research investigating these mechanisms is warranted.

Contribution of intestinal ceramides to whole-body metabolic dysfunction

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Introduction: Circulating ceramides, a family of lipid molecules composed of a sphingosine and a fatty acid, are modulated by diet and influence the risk, incidence and/or severity of metabolic diseases. The precise tissue of origin that dietary ceramides are derived from is currently thought to be the liver, but as the small intestine is the first to receive dietary lipids and export them into the circulation via the lymphatics, we hypothesised the gut-lymph axis of ceramide synthesis and export could be responsible for regulating ceramides in the circulation and that gut-derived circulating ceramides contribute to metabolic dysfunction[1]. The aim of this study was, therefore, to investigate how gut-derived ceramides contribute to metabolic dysfunction and to determine if targeting ceramide synthesis in the gut could be a new therapeutic strategy for metabolic disease.
Methods: Male Sprague Dawley rats were fed a control, or high-fat diet (HFD; 43% calories from lipids) for 7 weeks. At study endpoint, the efferent mesenteric lymphatic duct was cannulated in rats to assess the lipidomic signature within the lipid-rich core of lymph-derived chylomicron particles. We, next, generated a transgenic mouse to conditionally overexpresses the *Ceramide Synthase 2* (*CerS2*) enzyme in the intestinal epithelium upon chemical induction with tamoxifen. Littermate controls and mice heterozygous for the *CerS2* transgene received a control, or HFD for 4 weeks, during which time glucose and lipid metabolism were assessed pre- and post- tamoxifen induction, which resulted in the activation of *CerS2* overexpression. Tissues were collected at 16-17 weeks of age for lipidomic and metabolic analysis.
Results: Whole-mesenteric lymph and chylomicron particles from rats were enriched in sphingolipids, of which metabolically toxic shorter-chain ceramide species were elevated in HFD-fed animals. Epithelial cells lining small intestinal segments, which are responsible for lipid absorption, exhibited a similar distribution of ceramides mirroring the profile of the chylomicrons, and high-fat feeding further increased specific ceramide species. HFD-fed mice with an intestinal overexpression of *CerS2* demonstrated increased long-chain ceramide synthesis and decreased toxic $C_{16:0}$ ceramide production in the gut epithelium, leading to improvements in whole-body glucose metabolism and hepatic steatosis.
Conclusion: Intestinal derived ceramides contribute to whole body metabolic disease and could be a promising new therapeutic target.

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Dysregulation of amino acid and sphingolipid metabolism in co-morbidities of diabetes

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Metabolism is central to virtually all cellular functions and contributes to a range of diseases. A quantitative understanding of how biochemical pathways are dysregulated in the context of diseases such as cancer, metabolic syndrome, and neuropathy is necessary to identify new therapeutic targets. To this end we apply stable isotope tracers, mass spectrometry, and metabolic flux analysis (MFA) to explore metabolism in cells, animal models, and human patients. We are particularly interested in understanding how amino acid and lipid metabolism are coordinated in the context of cancer and diabetes. Serine, glycine and one carbon metabolism is critically important for cell function and health, but the amino acids associated with this pathway are commonly reduced in patients with metabolic syndrome. Here I will detail how we apply MFA and related methods to decipher why serine and glycine are reduced in mouse models of diabetes. At the same time, restricting dietary serine and glycine in a high-fat diet promotes neuropathy in C57BL/6 mice. In turn, supplementation of serine improves sensory function in diabetic animals, suggesting potential therapeutic strategies for treating patients with serine-associated neuropathy. We also provide evidence that fatty acyl-CoA diversity in sphingolipid biosynthesis impacts trajectories to insulin resistance/obesity and cardiotoxicity in mice. Collectively, these data provide mechanistic insights into the roles of serine palmitoyltransferase, amino acid metabolism, and fatty acid diversity in driving co-morbidities of diabetes.

Human pluripotent stem cell models reveal the mechanism of alpk3-induced cardiomyopathy

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Striated muscle contraction relies on a molecular system composed of specialized cytoskeletal proteins known as the sarcomere. Genetic mutations in genes encoding these sarcomeric proteins can instigate cardiomyopathy, a heart condition characterized by abnormal heart muscle growth and function, leading to heart failure and death. However, it is increasingly recognized that genetic mutations in genes unrelated to traditional sarcomeric proteins significantly contribute to cardiomyopathy. One such gene is ALPK3, responsible for an atypical alpha kinase. Despite its strong association with cardiomyopathy, its precise molecular function remained undefined. Thus, our study sought elucidate the molecular role of ALPK3 in health and disease. To achieve this, we generated a suite of gene-edited human pluripotent stem cells (hPSCs) and mice containing endogenous fusion proteins, loss-of-function mutations, or disease-causing patient variants. Our research demonstrated that ALPK3 is localized within the sarcomere of heart cells and revealed its interactome and phosphoproteome. ALPK3 deficiency impaired muscle cell function in both hPSC cardiac organoids and mice carrying a pathogenic ALPK3 variant. The ALPK3-dependent phosphoproteome and interactome were enriched with sarcomere and protein quality control proteins, including the ubiquitin-binding protein SQSTM1 and ubiquitin ligases. Notably, SQSTM1 exhibited mislocalization from the sarcomere in three independent ALPK3 patient lines. Our data collectively underscores that ALPK3 plays a pivotal role in regulating the connection between the sarcomere and the protein surveillance system and that its deficiency impairs cardiac function via this mechanism.

Phosphoinositide 3-kinase protects against atrial enlargement fibrosis and thrombi

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Phosphoinositide 3-kinase (PI3K, p110α: class 1A) is a critical regulator of exercise-induced ventricular enlargement and protection, but the role in the atria was unknown. In an unexpected observation we identified atrial enlargement, fibrosis and thrombi in a subset of cardiac-myocyte specific transgenic (Tg) mice with reduced PI3K. Understanding key mechanisms underlying atrial enlargement and pathology is important because they predispose to atrial fibrillation. Our previous work showed that cardiac-specific heterozygote Tg mice with reduced PI3K activity, due to the expression of a dominant-negative PI3K (dnPI3K) mutant on one allele (dnPI3K Tg(+/-)), had smaller hearts with normal heart function under basal conditions. When we unintentionally generated homozygote dnPI3K Tg mice (dnPI3K Tg(+/+)) due to a breeding error, we found atrial enlargement and thrombi in a subset of dnPI3K Tg(+/+) mice. In this presentation, I will describe the physiological and molecular phenotype underlying atrial myopathy in dnPI3K homozygote mice, and discuss the potential relevance in patients with atrial fibrillation.

Defective lysosome reformation during autophagy causes skeletal muscle disease

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Autophagy is an evolutionarily conserved, catabolic process that is essential for survival. It is responsible for the removal of intracellular debris including damaged organelles such as mitochondria. Skeletal muscle is amongst the tissues most reliant on the cytoprotective role of autophagy. The suppression of autophagy in muscle contributes to poor post-exercise recovery, age-related muscle decline (sarcopenia) and in severe cases, fatal inherited diseases called muscular dystrophies and myopathies. Therefore, muscle health is reliant upon maintaining a high rate of autophagic flux, even under basal conditions. However, this can be further elevated as an adaptive response to physiological stimuli including exercise or fasting, the latter to mobilize amino acids. Lysosomes play an integral role in autophagic degradation and are heavily utilized during this process. For autophagy to continue lysosomes must be replenished and the prevailing paradigm was that this occurs via *de novo* lysosome formation. However, in a new discovery we have shown that lysosome generation during muscle autophagy is dependent upon a complex membrane recycling process called "*autophagic lysosome reformation"* (*ALR*). Here, organelle membranes already formed during autophagy are recycled for use in efficiently generating the lysosomes needed for sustained muscle autophagy function. We define a novel mechanism for controlling lysosome reformation, via the spatiotemporal regulation of lipid signaling molecules called phosphoinositides. In an ingenious design, this process is also controlled by mTOR reactivation during autophagy, enabling cells to fine-tune the extent of lysosome reformation with the extent of autophagic flux. Using mouse knockout studies, we show how this pathway maintains muscle health *in vivo*, and how the failure of this process causes muscular dystrophy in patients. When ALR fails, lysosomes become depleted causing a marked autophagy defect that leads to severe muscle disease. In addition to highly deleterious effects on autophagy, this lysosome depletion is also likely to have broader effects on other lysosome-dependent functions, thereby causing severe disease. We are currently exploring how the failure of ALR impacts other critical muscle processes including mitochondrial respiratory function. This represents a significant advance given it is a new disease pathway in muscle, and as such, a strong possibility that it has been overlooked for other muscular dystrophies/myopathies. Our study also redefines the regulatory processes that control autophagy-related muscle health.

Targeting central feeding circuits to improve outcomes in cancer cachexia

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(introduced by Dr. Adam Hagg, University of Queensland)

Introduction: Cachexia is a progressive loss of body weight, accompanied by loss of appetite, which affects as many as 80% of cancer patients. The current focus of anti-cachexia research is blockade of tumour-derived circulating factors at the level of fat and muscle. However, given that the brain is the master regulator of metabolic control, targeting the brain to alter metabolic outcomes in cachexia is an attractive idea. Ghrelin is a peptide hormone which rises in response to fasting, drives eating behaviour, decreased energy expenditure and growth hormone release. The primary target neurons of ghrelin are the Agouti related peptide/neuropeptide Y-containing neurons in the arcuate nucleus of the hypothalamus (AgRP neurons). Therapies using ghrelin analogues have been trialled as a way to target the brain to treat cachexia.

Methods/Results: We used a mouse model of pancreatic ductal adenocarcinoma (PDAC) to assess ghrelin action *in vivo*. After the onset of PDAC-induced anorexia, PDAC-carrying mice ate significantly less than control mice in response to injected ghrelin, indicating ghrelin loss of ghrelin sensitivity in cancer cachexia even before noticeable wasting has occurred. These mice showed elevated levels of LEAP-2, an endogenous antagonist for the ghrelin receptor, indicating perturbations in ghrelin physiology.

To circumvent the observed ghrelin resistance, we used targeted chemogenetics (DREADDs) to chronically artificially activate AgRP neurons during cancer cachexia in PDAC-bearing mice by using an AAV to introduce the hM4Dq mutant receptor transgene specifically into AgRP neurons. This was performed via stereotaxic surgery under isoflurane anaesthesia (2% in oxygen). Activating AgRP neurons via the hM4Dq DREADD with the selective ligand clozapine n oxide (CNO) rescued both fat and skeletal muscle mass loss in male mice. This effect was dependent on the increased feeding induced by activating AgRP neurons. We measured circulating levels of the pro-cachexia factors, activin A and B. PDAC-bearing mice with or without AgRP neuronal activation showed a similar, significant elevation in activin A and B levels, compared to non-PDAC bearing mice. Importantly, AgRP neuronal activation protected mice from the wasting effects of elevated activins, as the levels seen in this model are sufficient to drive significant wasting.

Conclusions: This provides the first evidence that direct activation of AgRP neurons drives muscle and fat retention in cancer cachexia, and opens the door to therapies that exploit central mechanisms for energy balance control.

Unveiling a Hidden Signaling Landscape: Profiling the Secretome of the Medial Basal Hypothalamus in Metabolic Disease

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Metabolic diseases such as obesity and type 2 diabetes are driven by intricate cellular communication pathways, involving the secretion of proteins and peptides acting through autocrine, paracrine, and/or endocrine mechanisms to perpetuate disease. Within the mediobasal hypothalamus (MBH), a crucial regulator of metabolism, peptide and protein secretion is orchestrated in response to nutritional status and obesity. While research has primarily focused on MBH-secreted neuropeptides derived from pro-opiomelanocortin (POMC), such as α-Melanocyte-stimulating hormone, the broader landscape of MBH secretory molecules from both neuronal and non-neuronal cells within the MBH remains largely uncharted.

To address this knowledge gap, we developed a pioneering methodology for identifying the secretory peptidome and proteome of the MBH in conscious, freely behaving mice. This innovative approach involves the precise placement of custom-built ultrabroad capture micro-dialysis probes (with a molecular weight cutoff <1,000,000 Da) and real-time, µL-resolution sampling for unbiased peptide and protein sequencing via LC-MS/MS.

We surgically implanted guide cannulas above the MBH in male C57Bl/6 mice subjected to a high-fat diet (HFD) or a standard chow diet for up to 12 weeks. After a 2-week post-implantation period, mice were fasted overnight, and microdialysis probes were inserted into the MBH to collect secretory dialysate for 2-hours in the fasting state. Subsequently, mice were allowed *ad libitum* access to food for 2 hours, during which fed dialysate was collected. Tandem mass tag (TMT) labelling, coupled with mass spectrometry-based proteomics and peptidomics, enabled us to comprehensively uncover the MBH secretome.

Our analysis unveiled 2,148 proteins and 1,426 peptides within the MBH secretome, including the identification of previously unknown peptides. Notably, our peptidomic analysis revealed that fasting suppressed POMC neuropeptide expression in chow diet mice, which was dysregulated in obesity. This corroborates findings from numerous histological studies and reaffirming the sensitivity and relevance of our method. Additionally, we identified a subset of peptides differentially regulated by fasting and others altered in the context of obesity. Collectively, our innovative discovery pipeline has shed light on the intricate secretory profile of the MBH, revealing a previously underappreciated complexity in cellular communication. These findings hold promise for identifying novel therapeutic targets for the treatment of metabolic diseases.

Mapping the diversity of neuronal populations in the inner ear

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The heterogeneity of neuronal types in the auditory and vestibular afferent systems is matched to the complexity of primary sensory encoding. The two major types of auditory primary afferent neurons are long-established, with type-I spiral ganglion neurons (SGN) synapsing with cochlear inner hair cells and type II SGN synapsing with outer hair cells. With advancements in single cell RNA sequencing, further heterogeneity of type-I SGNs was determined, solidifying type-Ia, Ib and Ic as molecularly and functionally distinct populations (Sun, *et. al*., 2018). In the vestibular ganglion, large calyx afferent synapse with type-I hair cells, while small bouton afferents synapse with type-II hair cells (Leonard, and Kevetter*,* 2002).
Investigating inner ear neuronal populations in transgenic reporter models has expanded the diversity of these afferent neurons, where for example the intermediate filament protein Peripherin (Prph) is a known marker of type-II auditory neurons and vestibular bouton afferents. Using the regulatory elements of the Peripherin promoter, we developed a construct with conditional ablation (hDTR) and mCherry fluorescent labelling reporter elements ($Prph_p$-hDTR-mCherry). This construct was delivered into the male pronucleus of C57Bl/6J mice, leading to establishment of a homozygous line. All mice in this study were used with approval of the UNSW Animal Care & Ethics Committee.
To resolve intact inner ear afferent populations, we developed a CUBIC1/PEGASOS clearing, light sheet Z1 imaging and Imaris segmentation pipeline. Mapping the distribution of mCherry positive neurons throughout the inner ear delineated a population of SGN extending from the base (high frequency encoding region), and in small diameter neurons of the vestibular ganglion. There was significant mismatch between the mCherry positive SGN and native Peripherin SGN expression, suggesting the mCherry+ population marked cells that are a mixed macro-class of type-II and type I auditory neurons.
Nanopore sequencing was used to assess the whole mouse genome, identifying the $Prph_p$-hDTR-mCherry transgene integrated within the gene G*rm8* (mGLUR8). It is likely this integration influenced the transgene expression phenotype. *Grm8* is an SGN subtype marker, exhibiting elevated expression in type-Ic neurons (modiolar facing and low spontaneous firing). The mCherry+ neurons show most prominent overlap with type-Ic marker *Pou4f1* (BRN3A) (Sun, *et. al*., 2018). Although this mCherry population shows a mixed profile, the high frequency localisation and increased overlap of mCherry+ neurons with type-II and type-Ic auditory neurons, suggests there may be physiological implications of this primary afferent population in hearing. Patch clamping, hearing testing and balance studies offer pathways of future investigation to uncover the single cell and population function of the $Prph_p$-hDTR-mCherry auditory and vestibular neuron populations.

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Sarcopenia, the dark side of ageing: the rise of bone metabolism

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The loss of muscle mass, strength and function associated with ageing is known as sarcopenia. Sarcopenia is associated with increased risk of falls, fractures, diabetes, cardiovascular disease all can lead to a reduction in quality of life and premature mortality. To date, there is no effective drug treatment for sarcopenia.

Recently, bone was recognised as an endocrine organ, with several bone-derived factors suggested to be involved in the maintenance of skeletal muscle mass and strength, as well as, muscle metabolism. Research efforts over the last decade predominantly focused on bone turnover markers (BTMs) predominantly osteocalcin (OC), however, other circulating bone-related factors such as the receptor activator of nuclear factor-kB ligand (RANKL), osteoprotegerin (OPG), sclerostin (SCL) and lipocalin-2 (LCN2) may be involved. This presentation will focus on the potential role bone might play in future targeted treatments for sarcopenia and its associated conditions including insulin resistance and type 2 diabetes.

Development of RyR2 inhibitors and verification of their effects in CPVT mouse models

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Hyperactivation of type 2 ryanodine receptor (RyR2) has been implicated in ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) and heart failure, where spontaneous Ca^{2+} release via overactivated RyR2 depolarizes diastolic membrane potential to induce triggered activity. Conventional antiarrhythmic drugs, i.e., Na channel inhibitors, Ca channel inhibitors, ß-blockers, used to treat CPVT sometimes fail to suppress arrhythmias, and implantable cardioverter defibrillators (ICDs) could exacerbate arrhythmias. Reducing RyR2 activity is thought to suppress arrhythmias in CPVT, but there are no clinically available antiarrhythmic drugs with RyR2-specific inhibitory action. We searched for RyR2 specific inhibitors by a high-throughput screening and found several RyR2 inhibitors. Some of the hit compounds suppressed Ca^{2+} waves and sparks without affecting action potential-induced Ca^{2+} transients in isolated cardiomyocytes. We further developed a high-affinity and selective RyR2 inhibitor based on one of the hits. This compound successfully suppressed arrhythmias in CPVT mouse models harboring mutant RyR2s. In contrast to conventional anti-arrhythmic drugs, this compound did not affect ECG parameters at the effective doses. Our results suggest that the RyR2 specific inhibitor may represent a new category of anti-arrhythmic drugs.

Piezo1 is the cardiac mechanosensory that initiates the cardiomyocyte hypertrophic response to pressure overload in adult mice

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Pathological left ventricular hypertrophy (LVH) is a maladaptive response of the LV myocardium to increased cardiac load. It causes an increase in LV wall thickness driven by enlarged individual cardiomyocytes and is accompanied by fibrotic remodelling. So far, the treatments are limited and none of the currently available interventions can completely reverse the pathological effects on the myocardium once LVH is established. Therefore, a better understanding of the mechanisms responsible for pressure overload induced LVH may open new therapeutic avenues. We had previously reported that the transient receptor potential melastatin 4 (TRPM4) ion channel plays a role in pressure overload induced LVH via activation of the Ca2+-dependent kinase CaMKII. However, TRPM4 is neither stretch-activated nor Ca^{2+}-permeable. Despite the significant evidence for a role of Piezo1 channels, which is both stretch-activated and Ca^{2+}-permeable in vascular physiology and pathophysiology, its role in the heart is largely unknown.
The purpose of this study was to determine; (1) whether cardiomyocyte Piezo1 influences LVH, (2) whether Piezo1 activation in response to pressure overload results in CaMKII activation, and (3) how TRPM4 participates in this activation.
Using mice expressing a Piezo1 fusion protein (Piezo1-tdTomato) and a cardiomyocyte-specific inducible knockout (Piezo1 KO) mouse model, we induced LVH by pressure overload in response to transverse aortic constriction (TAC). We demonstrate that in response to TAC-induced pressure overload, cardiomyocyte expression of Piezo1 increases, and that cardiomyocyte-specific deletion of Piezo1 in adult mice prevents activation of CaMKII-HDAC4-MEF2 pathways and inhibits the LVH observed in response to pressure overload induced by TAC. Moreover, we show that Piezo1 not only colocalizes with TRPM4 in cardiomyocytes but also that these channels physically interact, providing a basis for their functional coupling. Loss of Piezo1 prevents the altered expression of several critical Ca^{2+} handling proteins, including TRPM4, and the sodium-calcium exchanger (NCX), that is associated with pressure overload-induced LVH.
We concluded that Piezo1 is the cardiomyocyte mechanosensor that transduces the increased myocardial forces caused by pressure overload into a chemical signal that activates the hypertrophic signalling cascade resulting in pathological LVH. Thus, the Piezo1-TRPM4 signalling cascade may represent a novel therapeutic target for LVH.

Regulation of a Novel Splice Variant of Early Growth Response 4 (EGR4-S) by HER+ Signalling and Heat Shock Factor 1 in Breast Cancer

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INTRODUCTION: Research shows that elevated cellular stress can accelerate cancer progression and that metastatic tumours show a global increase in stress response compared to normal tissues. One response to cellular stress, called the Heat Shock Response, is triggered by many forms of cell stress. The 'master regulator' of the Heat Shock Response is the transcription factor Heat Shock Factor 1 (HSF1), which is activated when a cell is under stress. Our previous work identified that the gene Early Growth Response 4 (*EGR4*) is regulated by HSF1 in human breast cells. EGR4 is a transcription factor known to play an important role in the proliferation of small cell lung cancer. Given that HSF1 and cell stress play a role in cancer progression, metastasis, and drug resistance, and our previous finding that *EGR4* is regulated by HSF1 in human breast cells, the aim of this study was to investigate *EGR4* as a potential oncogene in breast cancer and its regulation by HSF1.
METHODS: Commercially available breast cancer cell lines (n=12) were analysed by Western blot and quantitative polymerase chain reaction (qPCR) to determine EGR4 and HSF1 expression levels. Cell lines were subsequently modified (HSF1 knockdown and overexpression) to examine the effect on EGR4. Additionally, anti-cancer drugs (lapatinib, gefitinib, erlotinib) were administered to examine their effect on EGR4 and HSF1 expression. Human breast tumour biopsies (n=28) were also analysed to validate findings from cell culture.
RESULTS: Our research identified a new, shortened version of the EGR4 protein (which we named EGR4-S), found in breast cancer but not detectable in normal breast tissue. Interestingly, our findings show that the EGR4-S expressed by breast cancer cells could be reduced by treating the cells with certain targeted cancer therapeutics. However, sustained, high-dose treatment led to EGR4-S becoming less responsive. In addition, we identified an inverse relationship between EGR4-S and cellular stress. When cancer cells were in conditions of increased cellular stress, reduced EGR4-S levels were associated with lower growth rate but enhanced properties associated with higher metastatic potential.
CONCLUSION: Taken together, our research suggests further investigation of EGR4-S is warranted in order to determine its potential as a biomarker for differentiating tumours from normal tissue at the molecular level, as well as its possible resistance to targeted therapies.

LPS-induced inflammation disrupts Ca^{2+} homeostasis and differentially impairs contractile function in respiratory and limb muscles of mice.

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Skeletal muscle weakness and wasting with an inflammatory component is observed in multiple diseases including COPD, congestive heart failure and sepsis (Reid et al., 2002). Although the mechanisms underlying inflammation induced skeletal muscle weakness are not well understood, increased production of reactive oxygen species (ROS) and impaired Ca2+ handling have been implicated (Liu et al., 2002). A better understanding of the interaction between inflammation, ROS and Ca^{2+} handling could identify strategies to improve patient outcomes for inflammatory related pathologies. Neutrophil-derived ROS have previously been suggested as a cause of inflammation-induced skeletal muscle weakness following intraperitoneal (IP) injections of lipopolysaccharides (LPS) (Supinski et al., 1999). This relationship has only been confirmed in the diaphragm, but it remains unclear whether LPS has a global effect on skeletal muscle function. Furthermore, it is not known how LPS impacts excitation contraction coupling. The aim of this study was to determine the effects of LPS on intracellular Ca^{2+} handling and contractile function in limb and respiratory muscles.

Twelve-week-old male C57BL/10 (C57) mice were injected IP with either 12.5 mg/kg LPS (*Escherichia coli* 0111:B4, Sigma Chemical, St. Louis, MO) or isotonic saline (Sal). Twenty-four hours after injection, diaphragm muscle fibres and extensor digitorum longus (EDL) muscles were surgically removed from anaesthetized Sal and LPS mice (IP 40 mg/kg sodium pentobarbitone). Muscle preparations were mounted in an *in vitro* muscle test system (1200A, Aurora Scientific, Canada) and evaluated for contractile function. Isolated interosseous muscles were digested with 0.2% collagenase at 37 °C to obtain single fibres. Fibres were loaded with a Ca^{2+} sensitive dye (10 μM FURA-2, AM ester) and imaged at rest and during a twitch contraction (Nikon 'S Flour 1.30 oil immersion', Japan). Diaphragm and quadrilateral EDL samples were snap frozen in liquid nitrogen and stored at -80 °C for subsequent analysis of reversible thiol oxidation (a sensitive measure of ROS mediated oxidation) and neutrophil activity, as measured by muscle myeloperoxidase (MPO) activity. Data were analysed using unpaired t-tests or 2-way ANOVAs where appropriate.

Maximum specific force was 39% lower in the diaphragm of LPS treated mice compared to controls, and twitch force was 52% lower ($p < 0.001$). In contrast, neither maximum specific force nor twitch force of the EDL were significantly affected by LPS. Both muscles however showed significant differences in twitch time parameters after LPS exposure, including increased half relaxation time (by 41% in the EDL; $p < 0.001$), and decreased maximum and minimum dF/dt (by 38% and 43% respectively in the diaphragm; $p < 0.01$). Resting intracellular Ca^{2+} was 102% higher, and Ca^{2+} transient amplitude 49% lower in interosseous fibres after LPS exposure compared to controls ($p < 0.001$). Analysis of frozen tissue samples revealed that both MPO activity and thiol oxidation of diaphragm muscles were significantly increased by LPS exposure (by 96% and 13% respectively, $p < 0.05$). Interestingly, there was no effect of LPS in either measure on EDL muscles.

These results confirm that IP LPS exposure significantly impairs contractile function in isolated skeletal muscle and these effects may be mediated by alterations to Ca^{2+} handling. Those effects appear to be mediated by neutrophil-derived ROS in the diaphragm, but not in the EDL. Overall, these findings suggest that the diaphragm is affected by IP LPS injections more severely than limb muscles. This differential effect may be due to the closer proximity of the diaphragm to the injection site which may have induced a local inflammatory response in addition to the systemic effects on the limb muscles. Further tissue analysis will be performed to determine if the effects recorded in the limb muscles are mediated by cytokines, like TNF-α, disrupting Ca^{2+} homeostasis.

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Hypothalamic Neurons Expressing the Relaxin Receptor, Rxfp1, Orchestrate Feeding Behaviour and Glycaemic Control for Metabolic Disease Remission in Mice

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Neuronal populations residing within the arcuate nucleus of the hypothalamus (ARC) play a pivotal role in governing whole-body energy expenditure and glucose homeostasis. Dysfunction of ARC neurons is a key contributor to metabolic disorders including obesity and type-2 diabetes. While the conventional perspective implicates well-studied ARC neuronal populations expressing agouti-related peptide/ neuropeptide Y (AgRP/NPY) or pro-opiomelanocortin (POMC), considerable evidence challenges this dogma, including the heterogeneity and potential functional redundancy of these neurons in metabolic regulation. Consequently, we explored the possible existence in mice of previously unidentified neuronal populations within the ARC that are crucial for energy balance and glycaemic control.

In these studies, we detected the presence of relaxin-family peptide receptor-1 (Rxfp1)-expressing neurons within the ARC and throughout the mouse brain; and employing dual-reporter mouse models (*Rxfp1*-Cre;LSL-tdTomato;*Pomc*-GFP and *Rxfp1*-Cre;LSL-tdTomato;*Npy*-GFP), we conclusively demonstrated that Rxfp1-positive neurons constitute a distinct population separate from canonical AgRP/NPY and POMC neurons. Single-cell droplet sequencing of ARC samples further validated the unique identity of Rxfp1-positive neurons, which exhibit a specific molecular signature (67.9% Tac, 5.11% Anxa2, 4.26% Ttr, and 22.73% other).

In viral-based studies to elucidate the functional role of Rxfp1 neurons within the ARC, we employed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) for precise, on-demand neuronal activation. Specific stimulation of Rxfp1 neurons led to hypophagia, altered whole-body carbohydrate/fat utilisation, and improved glycaemic control.

In pursuit of pharmacological interventions, we centrally administered a newly developed and highly selective Rxfp1 agonist to diet-induced obese mice. Daily dosing promoted a dose-dependent reduction in food intake and body weight, accompanied by a marked enhancement in glycaemic control. Collectively, our findings identify, characterise, and pharmacologically target a specific neuronal population within the ARC that offers promise for the remission of metabolic diseases.

Targeting AMPK to improve survival in a Colon-26 mouse model of cancer

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Cancer is a leading cause of death worldwide. Metabolic dysfunction is a hallmark of cancer that contributes to patient morbidity and mortality. Therapeutic approaches to modulate the systemic cancer environment, while restoring metabolic function, should improve survival and physical function in cancer patients. Small-molecule, allosteric activators that directly bind to 5' adenosine monophosphate-activated kinase (AMPK), a key regulator of cellular energy homeostasis, have been shown to improve glucose homeostasis in diabetes (Myers *et al*., 2017) and may have therapeutic potential in cancer. The purpose of this study was to determine the therapeutic efficacy of the pan-AMPK activator, MK8722, in a mouse model of colon cancer.

All animal experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (NHMRC). We examined the *in vitro* effects of MK8722 (MedChemExpress, USA) on colon-26 (C-26) cell proliferation (cell counts), mitochondrial function (Agilent Seahorse XF), and growth-related signalling pathways (through western blotting). Male BALB/c mice (11-12 weeks of age) (n=60) were anaesthetised (2-5% isoflurane), and the right flank shaved and injected with C-26 cells (5×10^5 cells/mouse, *s.c.*) or with phosphate buffered saline. Mice were treated daily with MK8722 at 0, 1 or 10 mpk (n=8-12/group, *i.p.*) beginning on day 5 until humane endpoint criteria, or until day 30, was reached. At endpoint, mice were anaesthetised (sodium pentobarbitone, 60 mg/kg, *i.p.*) and killed as a consequence of cardiac excision. Tumour *Vegf* gene expression was measured by quantitative PCR. Data were compared using a one-way ANOVA with Bonferroni's post hoc test.

MK8722 (0.1, 1 and 10 μM) increased AMPK activity in C-26 cells, resulting in a dose-dependent reduction in cell proliferation (p<0.01), basal and maximal oxygen consumption rate (p<0.01) and growth-related signalling (p<0.0001). Untreated C-26 tumour-bearing mice began losing body mass at day 8 which continued throughout the study period. In contrast, treated C-26 mice demonstrated a dose-dependent preservation of body mass at endpoint (p<0.0001), and improved survival with the high dose (10 mpk) MK8722 treatment (p<0.05). There were no differences in tumour volume or growth rate with MK8722 treatment, but *Vegf* gene expression was decreased (p<0.01) at the high dose, suggesting potential effects on tumour angiogenesis.

Together, these findings suggest the pan-AMPK activator MK8722 can promote survival in C-26 tumour-bearing mice in a dose-dependent manner. Additionally, we provide evidence that MK8722 may modulate the tumour environment independent of an anti-cancer effect. Further studies are warranted to determine whether MK8722 can alter tumour and/or muscle metabolism and whether this impedes cancer progression.

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Interrogating skeletal muscle plasticity in response to growth and oxidative stimuli

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An inverse relationship exists in skeletal muscle between fibre size and oxidative capacity with slow oxidative fibres typically being smaller and fast glycolytic fibres being larger. A highly conserved relationship among vertebrates is that muscle fibres hypertrophy at the expense of their oxidative capacity. The estrogen-related receptor family (Esrra, Esrrb, Esrrg) are established regulators of the slow oxidative phenotype, whereas follistatin (FST) is a potent mediator of muscle growth. The purpose of this study was to interrogate the adaptive potential of skeletal muscle to oxidative (Esrrg) and growth (FST) stimuli at different stages of development.
All experiments were approved by the Animal Ethics Committee of The University of Melbourne and Monash University and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). C2C12 myoblasts were transfected with control (CMV:EGFP), Esrrg (CMV:Essrg), FST (CMV:FST317), or Esrrg+FST plasmids (0.5 µg DNA/well) and differentiated for 5 days. Myotubes were stained for myosin heavy chain and myotube diameter was assessed (3 experimental replicates, 3-6 technical replicates per experiment). Wild-type zebrafish embryos were collected and microinjected with control (actc1b-mCherry), Esrrg (actc1b-Esrrg-IRES-mOrange), FST (actc1b-FST317-IRES-EGFP), or Esrrg+FST (750 ng/µl DNA, 300 µg/µl transposase mRNA) at the one-cell stage of development. Zebrafish were embedded at 6 days post fertilisation, imaged laterally by confocal microscopy, and diameter was assessed in myofibres positive for mCherry, mOrange, and/or EGFP (4 experimental replicates, 10-30 technical replicates per experiment). Male C57BL/6 mice (10-12 weeks of age) were anaesthetised (2-4% isoflurane) and received bilateral tibialis anterior (TA) muscle injections of recombinant adeno-associated viral serotype 6 (rAAV6) vector containing either: 1) MCS control; 2) Esrrg; 3) FST; and 4) Esrrg+FST (n=4-8/group/dose; ~4e9 vg/muscle). The left leg was injected with MCS, while the right leg was injected with the genes of interest. After 4 weeks of overexpression, mice received an intraperitoneal injection of pentobarbitone sodium (150 mg/kg) and were killed via cervical dislocation. Muscles were collected for assessment of myofibre diameter. Data were compared using a one-way ANOVA with Tukey post hoc test.
Esrrg overexpression promoted formation of smaller myotubes (-20%; $p<0.01$), whereas FST overexpression promoted formation of larger myotubes (+13%; $p<0.05$). Combined overexpression of Esrrg and FST resulted in the formation of smaller myotubes (-22%; $p<0.01$), suggesting that during myogenesis, *in vitro* oxidative programming can impede hypertrophic growth of C2C12 muscle cells. Similarly, Esrrg overexpression promoted formation of smaller myofibres in zebrafish (-43%; $p<0.05$), whereas FST overexpression promoted formation of larger myofibres (+32%; $p<0.05$). The combined overexpression of Esrrg and FST resulted in formation of smaller myofibres (-45%; $p<0.0001$), suggesting that during muscle development and maturation *in vivo*, oxidative programming can impede hypertrophic growth in zebrafish. Lastly, we assessed the plasticity of adult skeletal muscle to Esrrg and FST overexpression. Esrrg overexpression did not affect muscle mass or myofibre size, whereas FST overexpression induced muscle and myofibre growth (+46%; $p<0.0001$). Esrrg overexpression did not impede FST-induced myofibre growth (+51%; $p<0.0001$), suggesting that in adult skeletal muscle *in vivo*, oxidative programming does not impede hypertrophic growth in mice.
These findings demonstrate that growth-related pathways are sensitive to an oxidative stimulus during muscle formation, development, and maturation. In contrast, in adult skeletal muscle, oxidative programming does not impede the growth response. This highlights how the timing of an oxidative or growth stimulus can dictate the adaptive remodelling of muscle phenotype, which may have important therapeutic implications for ageing and other muscle wasting disorders.

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Diet-induced gut dysregulation promotes obesity-driven NAFLD/NASH via gut liver axis.

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Background: Excessive dietary energy intake has emerged as a key driver of obesity, Type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver diseases (NAFLD) and the more severe nonalcoholic steatohepatitis (NASH) (1). Obesity results from an energy imbalance where energy intake exceeds energy expenditure. One area of energy imbalance that has been overlooked is the absorption/digestive efficiency of the gut. It is now apparent that certain dietary nutrients increase digestive efficiency and the consumption of foods rich in fat and sugar can promote gut inflammation to result in compromised gut integrity or "leaky gut" (2,3). When gut integrity is disrupted, gut-derived bacterial products and metabolites "leak" through to the liver to drive NAFLD/NASH.

Our aim is to identify which specific dietary nutrients, fats, sugars or a combination of both, alter digestive efficiency, induces gut permeability and contributes to liver injury during metabolic disease.

Methods: C57BL/6 mice were fed chow diet (CD 9% calories from fat), HFD (43% fat), WD (40% fat + 0.15% cholesterol), HFD plus Fructose (HFHFrD, 40% fat + 30% fructose drink) and HFD plus glucose (HFHGD, 40% fat + 30% glucose drink) for 16 wk. Mice were metabolically assessed for body weight changes, adiposity, oral glucose tolerance, digestive efficiency and "leaky gut". Intestinal segments and the liver were assessed for markers of inflammation and steatosis.

Results: Mice fed with high caloric diets (WD, HFD, HFDHFrD and HFDHGD) exhibited increased change in body weight due to adiposity and impaired glucose tolerance. All diet groups consumed a relatively equal number of calories, however, faecal caloric content was reduced and digestive efficiency increased in mice fed WD compared to CD. This suggests mice consuming the WD have increased intestinal nutrient absorption which is reflected by excessive hepatic steatosis. Experiments are ongoing to understand if specific high calorie diets are increasing intestinal inflammation, disrupting gut barrier and contributing to hepatic injury associated with NAFLD/NASH.

Conclusion: High caloric diets contribute to energy imbalance and obesity. Addition of cholesterol in WD influences intestinal nutrient absorption which additionally contributes to whole body metabolic dysfunction.

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Heat Shock Proteins in human single skeletal muscle fibres resist age associated alterations and differentially respond to high-intensity exercise training

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Heat shock proteins (HSPs) are ubiquitously expressed proteins that help preserve cellular homeostasis. Within mammalian skeletal muscle three of the better characterized HSPs are HSP72, HSP27 and αB-crystallin (ABC). Among other roles, these three HSPs are involved in regulation of muscle protein protection and turnover and may be upregulated in ageing. High-Intensity Interval Training (HIT) can improve muscle health and function in older-adults, but it is unclear whether HSP levels can be increased following training. We examined single muscle fibre segments from vastus lateralis muscle samples from young (25±3 years) and older-adults (70±4 years) healthy individuals. An additional sample was collected from older adults following 12 weeks of HIIT (4x4 min at 90-95% of peak HR, with 4-min active recovery at 50-60% peak HR). Results; The abundance of HSP70, HSP27, αβ-crystallin did not differ between young and older adults in Type I or Type II fibres. In Type I and II fibres there was increased abundance of pABCSer59 and pHSP27Ser82 in older adults compared to young adults. HIT abundance of HSP70 of HSP70 in Type I fibres, tended to decrease the abundance of HSP27, pHSP27Ser15, pABCSer59 in both fibres types. Thus, in healthy, older individuals, the basal levels of HSP72, HSP27, αβ-crystallin or pHSP27Ser82 are not different to those in young adults in either Type I or Type II fibres but did show higher amounts of phosphorylated sHSP. In adaptation to HIT in older adults there was a reduction in the level of sHSP phosphorylation making them more akin to fibres from young individuals. Showing that HIT training in older individuals can result in a younger heat shock protein motif at resting levels.

Interrogating the biological roles of dystrophin and utrophin in contraction-mediated adaptations to dystrophic skeletal muscle

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Duchenne muscular dystrophy (DMD) is a progressive and severe muscle wasting disease caused by mutations or deletions in the dystrophin gene, for which there is still no cure or effective treatment. In patients with DMD and in two well-characterised murine models deficient in dystrophin (*mdx*) or dystrophin and utrophin (*dko*), muscles are fragile, injury prone and compromised in their regenerative capacity. Having recently identified novel roles for dystrophin and utrophin in the metabolic remodelling of dystrophic skeletal muscle to chronic low-frequency electrical stimulation (LFS, 10 Hz, 350 μs pulse duration, 12 h/d, 28 d) (Hardee *et al*., 2021), we sought to determine how these membrane-associated proteins are implicated in mechano-metabolic signalling following muscle contraction.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). Wild-type (C57BL/10; n=12) and dystrophin/utrophin-deficient (*dko*; n=12) mice were anaesthetised with ketamine/xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and microelectrodes implanted surrounding the sciatic nerve to facilitate unilateral, wireless stimulation of the lower hind limb muscles. Mice were subjected to a single bout of LFS (10 Hz, 12 h) with the right leg being stimulated and the unstimulated left leg serving as the contralateral control. Total proteomics and phosphoproteomics (n=6 mice/genotype) were performed immediately post-stimulation, while quantitative PCR was performed 3 h post-stimulation.

Total proteomics identified 3,049 proteins dysregulated in non-stimulated muscles of *dko* mice. As expected, there was a decrease in proteins associated with the dystrophin-associated glycoprotein complex and compensatory upregulation of proteins associated with the integrin complex. Dystrophin/utrophin deficiency altered the expression of proteins involved in muscle contraction, metabolism, and translation in *dko* mice. No differences in the total proteome were observed immediately post-stimulation in both wild-type and *dko* mice. Phosphoproteomics was then performed to understand how these basal perturbations affected contraction-mediated signalling. A total of 1622 phosphosites (866 phosphoproteins) were significantly regulated by contraction in wild-type mice, while only 302 phosphosites (241 phosphoproteins) were significantly regulated in *dko* mice. Kinase-substrate enrichment analysis revealed activation of AMPKA1, Akt1, ERK2, PKACA, and mTOR after contraction in wild-type mice. In contrast, while AMPKA1 and Akt1 were activated in *dko* mice, dystrophin/utrophin deficiency impaired the activation of PKACA and mTOR and decreased the activity of ERK1, ERK2, and CDK1. Similar to our previous observations with chronic LFS, dystrophin/utrophin deficiency impaired the activation of metabolic genes (e.g., *Pdk4*, *Hk2*) post-stimulation in *dko* mice.

The findings reveal that the absence of dystrophin and utrophin disrupts mechano-metabolic signalling and the transcriptional regulation of metabolic genes and identifies novel biological targets for restoring adaptive remodelling to muscular contraction in DMD.

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Dysferlinopathy and myofibre-type specific differences: Further investigation of protein and functional changes in dysferlin-deficient muscles

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Dysferlinopathies are a clinically heterogeneous group of muscular dystrophies caused by gene mutations resulting in deficiency of the membrane-associated protein dysferlin. The disease typically manifests post-growth and primarily affects the muscles in the limb-girdle, arms, and legs. It is characterised by progressive skeletal muscle wasting, inflammation, accumulation of lipid droplets in slow-twitch myofibres, and, in later stages of the disease, replacement of myofibres by adipocytes. However, the mechanisms for this disease pathology are not well understood. Previously, we showed that dysferlin deficiency results in myofibre-type specific differences in the function of predominantly slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles, differences that could not be fully accounted for by altered myofibre-type composition between 10-month-old dysferlin-deficient BLAJ and C57BL/6J wild-type (WT) mice (Lloyd *et al.*, 2019).
To further investigate these functional differences, in this present study, we quantified levels of proteins related to calcium (Ca^{2+}) handling and glucose/glycogen metabolism, in soleus and EDL muscles (n = 6) from 10-month-old BLAJ and WT mice, contralateral to those used in Lloyd *et al.* (2019). We also assessed the Ca^{2+}-activation properties of isolated slow- and fast-twitch myofibres from BLAJ and WT mice aged 3 months (n = 2 mice). All mice were anaesthetised using sodium pentobarbitone (40 mg/kgBM, i.p.), soleus and EDL muscles excised, and then mice euthanised by overdose of pentobarbitone. For protein analyses, muscles were snap-frozen and underwent standard western immunoblotting procedures (Xu *et al.*, 2017; Lamboley *et al.*, 2021). For isolated myofibre experiments, muscles were pinned in paraffin oil and myofibres isolated from the medial region, then chemically skinned with Triton X-100, mounted in a force transducer system, exposed to a series of Ca^{2+}-buffered solutions of increasing Ca^{2+} concentrations (pCa >8-4.5), and myofibre-type (i.e., slow type 1 or fast type 2) identified using strontium.
Marked differences between BLAJ and WT whole muscles were evident for several proteins. The main differences in BLAJ slow soleus were increased abundance of calsequestrin-1 and decreased glycogen synthase proteins ($p < 0.05$). Whereas, for BLAJ fast EDL muscles, there were increased levels of proteins for dihydropyridine receptor-1 and sarco/endoplasmic reticulum Ca^{2+}-ATPase 1, and decreased glucose transporter type 4 and glycogen debranching enzyme ($p < 0.05$); additionally, for BLAJ EDL muscles, the abundance of RyR1 was below the level of detection for 5 of the 6 muscles assessed. For the isolated myofibres, there were no differences in the Ca^{2+}-activation properties of slow- and fast-twitch dysferlin-deficient myofilaments.
These results demonstrate the complex differential impact of dysferlin deficiency on Ca^{2+} handling and glucose/glycogen metabolism proteins in slow-twitch and fast-twitch muscles. These unusual alterations in protein abundances, without intrinsic changes in Ca^{2+}-activation of the contractile myofilaments, likely contribute to the subtle muscle-type-specific functional changes shown previously in BLAJ mice.

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Unravelling the role of deubiquitinase Ubiquitin-Specific-Protease-15 in skeletal muscle

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Post Translational Modification (PTMs) plays an important role in regulating protein function, interaction, localisation, synthesis, and breakdown in skeletal muscle. One of these modifications is ubiquitination, which involves the addition of a small molecule called ubiquitin onto the target protein via an E2 conjugating enzyme and E3 ligase complex. Deubiquitinases (DUBs), are the enzymes that cleave ubiquitin off target proteins. There are ~100 DUBs expressed in the human genome; however, their roles in skeletal muscle are poorly understood. In non-muscle tissue, the DUB, Ubiquitin-Specific-Protease-15 (USP15), has been shown to target members of the TGFβ signalling pathway (Inui et al., 2011) and the TGFβ signalling pathway is known to be a potent regulator of skeletal muscle. The aim of this study was to determine the role of USP15 in skeletal muscle under basal conditions and when TGFβ signalling is being activated or inhibited by Activin A (ActA) and Follistatin (Fst), respectively.

All animal experiments were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes as set out by the National Health and Medical Research Council Australia and were approved by the University of Melbourne Animal Ethics Committee. To understand the role of USP15 in skeletal muscle, we first studied the expression of endogenous USP15 in muscle undergoing wasting and growth, induced by intramuscular injection of recombinant adeno-associated viral vectors (rAAV) expressing ActA (rAAV:ActA) and Fst (rAAV:Fst), respectively, in the tibialis anterior (TA) muscle of healthy male C57Bl/6J mice (while anaesthetised under 2.5-4% isoflurane). We then used rAAV to overexpress and knockdown USP15 (shRNA targeting USP15) in basal healthy conditions and in muscle undergoing ActA-induced wasting and Fst-induced growth. All tissue was collected from mice terminally anaesthetised under 2.5-4% isoflurane.

The results showed that while endogenous USP15 protein did not change in muscle undergoing ActA-induced wasting, USP15 protein increased in muscle undergoing Fst-induced growth. Furthermore, USP15 overexpression or knockdown had no impact on basal skeletal muscle mass or rates of protein synthesis, but were associated with decreased or increased Lysine-48 (K48) ubiquitinated proteins, respectively. While overexpression and knockdown of UPS15 had no impact on ActA-induced muscle wasting, overexpression of USP15 potentiated Fst-induced muscle growth. The USP15-mediated potentiation of Fst-induced growth was not associated with an increase in rates of protein synthesis but rather a marked decrease in K48-ubiquitinated proteins, suggesting the possibility of reduced protein degradation.

Collectively, results have shown that while USP15 protein is elevated during Fst-induced muscle growth, USP15 does not play a role in regulating basal muscle mass in a healthy animal. Nonetheless, the overexpression of USP15 potentiates Fst-induced muscle growth, possibly due to a decrease in K48 ubiquitination and decreased protein degradation. Overall, this is the first study to offer insight into the role of USP15 in skeletal muscle homeostasis and under conditions of muscle atrophy and growth.

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Eccentric contractions, passive stretches and redox modulation increase nuclear translocation of mechanosensitive transcription factor yes-associated protein in fast-twitch skeletal muscle fibres

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Skeletal muscle shows a marked capacity to rapidly adapt to damaging forms of exercise such as those involving eccentric contractions as well as after oxidative damage (1). However, the mechanisms and signalling pathways involved in these processes are not fully understood. Yes-Associated protein (YAP) is a mechanosensitive transcription factor present in the cytoplasm of many cells, which upon activation, translocates into the nucleus leading to increased transcriptional activity of genes that promote cell proliferation, differentiation and repair (2). Therefore, YAP signalling could be playing a role in adaptation to exercise-induced damage and/or oxidative damage in skeletal muscle. To investigate this hypothesis, we examined whether damaging eccentric contractions, maximum isometric contractions, simple passive muscle stretches, and/or redox modulation were able to increase YAP nuclear translocation in fast-twitch skeletal muscle fibres.

Fast-twitch extensor digitorum longus (EDL) muscles were surgically isolated from ARC Swiss mice aged 6 – 10 weeks. All experimental work was approved by The University of Western Australia Animal Ethics Committee. Mice were anaesthetised via intraperitoneal (IP) injection of 40 mg/kg sodium pentobarbitone and subsequently euthanised via pentobarbitone overdose (> 120 mg/kg, IP) upon muscle removal. Isolated muscles were mounted in an *ex vivo* muscle test system (Aurora Scientific) and underwent a contractile protocol involving either: i) ten eccentric contractions (to 140 % of optimum length); ii) ten maximum isometric contractions iii) ten passive stretches (to 140 % of optimum length); or finally, H_2O_2 exposure (200 μM or 1 mM) while undergoing ten maximal Isometric contractions. All contractions were spaced 3 minutes apart. Controls received no contractile activation or stretch. After their respective contractile protocol, muscles were fixed in 4 % formaldehyde and myofibres were mechanically isolated and stained for YAP using immunofluorescence (with DAPI to identify nuclei). Myofibres were microscopically imaged (DeltaVision Elite), and images were subsequently analysed to identify and count the number of YAP positive nuclei (Nikon Imaging Software elements software). A nucleus was identified as a YAP positive nucleus if the YAP antibody fluorescence in the nucleus was brighter than the YAP antibody fluorescence outside the nucleus, with nuclear boundaries defined by DAPI staining.

No YAP nuclear translocation was observed in control myofibres or in muscles that had undergone 10 maximal isometric contractions (0 % YAP positive nuclei). However, adding passive stretches to the maximal isometric contractions to produce eccentric contractions increased YAP nuclear translocation (85 % YAP positive nuclei; $P < 0.0001$, one-way ANOVA). Passive stretches alone also increased nuclear YAP translocation (32 % YAP positive nuclei) compared isometric contractions ($P < 0.0001$, one-way ANOVA). Exposure of muscles to 200 μM H_2O_2 while undergoing maximal isometric contractions produced no YAP nuclear translocation. However, exposure of muscle to higher concentration of 1 mM H_2O_2 while undergoing maximal contractions resulted in an increased YAP nuclear translocation (64 % YAP positive nuclei; $P < 0.0001$, one-way ANOVA), which is different to YAP nuclear translocation induced by eccentric contractions ($P = 0.0313$, one-way ANOVA).

The results of this study indicate that damaging eccentric contractile activity and oxidants such as H_2O_2 can cause YAP nuclear translocation in fast-twitch skeletal muscle. Passive stretch alone is also able to moderately activate YAP nuclear translocation. These results identify YAP nuclear signalling as a potentially important mechanism in muscle fibre adaptation to eccentric contraction-induced stress and oxidative damage. Untangling the molecular mechanisms deployed during muscle adaptation to damaging stimuli is crucial to our understanding of the cycles of damage that occur musculoskeletal diseases and will be important in evaluating the actions of any therapeutic interventions.

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MyoD-family inhibitor proteins act as auxiliary subunits of PIEZO channels

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PIEZO channels are critical cellular sensors of mechanical forces. Native PIEZO1 channels can display nonuniform subcellular localization and exhibit different gating kinetics—principally, slower inactivation in many cell types when compared with heterologous expression systems. These observations could be explained by differences in lipid composition, curvature-dependent sorting or protein-protein interactions. Despite their large size, ubiquitous expression, and irreplaceable roles in an ever-growing list of physiological processes, few PIEZO channel-binding proteins have emerged. Here using affinity capture mass spectrometry in conjunction with fibroblast cell lines edited using CRISPR/Cas9 we found that MyoD family inhibitor proteins (MDFIC and MDFI), interact with both PIEZO1 and PIEZO2 channels. We confirmed using co-immunoprecipitation that these transcriptional regulators, bind to PIEZO1/2 channels and patch-clamp electrophysiology revealed they regulate channel inactivation. Using single-particle cryo-electron microscopy, we mapped the interaction site in MDFIC to a lipidated, C-terminal helix that inserts laterally into the PIEZO1 pore module. While the exact mechanism driving the biophysical changes induced by MDFIC/MDFI remains to be elucidated newer data implicates an annular lipid in this process. These PIEZO interacting proteins fit all the criteria for auxiliary subunits, contribute to explaining the vastly different gating kinetics of endogenous PIEZO channels observed in many cell types and shine light on mechanisms potentially involved in human lymphatic vascular disease.

The Ubiquitinomics of Skeletal Muscle Hypertrophy

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Ubiquitination is a post-translational modification that can regulate a protein's location, interactions, function and degradation. Ubiquitin is covalently bound to lysine (K) residues of a target protein by a complex containing an E2 ubiquitin-conjugating enzyme and E3 ubiquitin-ligase and is removed from target proteins by deubiquitinases (DUBs). Little is known about the extent and regulation of protein ubiquitination in skeletal muscle under different conditions. We previously performed the first quantitative analysis of the ubiquitinome in skeletal muscle undergoing atrophy induced by the upregulation of an E3 ubiquitin ligase [ASB2β; (1)]; however, to date, no studies have examined changes to the ubiquitinome during muscle hypertrophy. To this end, we performed a quantitative analysis of the ubiquitinome and total proteome in skeletal muscle undergoing rapid muscle growth induced by the TGFβ-ligand inhibitor, follistatin (FST).
All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Ed. NHMRC, Canberra). Eight-week-old male C57Bl/6 mice were anaesthetised with gaseous isoflurane and recombinant adeno-associated viral (rAAV) vectors, that express FST (or a control vector), were directly injected into the tibialis anterior (TA) muscle of the lower legs. After 7d, FST expressing, and control (Con), TA muscles were dissected from anaesthetised mice and snap frozen. Some muscles underwent processing for Western blot analysis, while others were processed for mass spectrometry-based quantitative analysis of the total proteome and ubiquitinome using 10-plex tandem mass tags as previously described (1).
Seven days of FST expression resulted in a 25% increase in TA muscle mass which was associated with altered phosphorylation of Smad2 (decreased), and Smad1/5/8 (increased). Moreover, muscle growth was associated with increased mTORC1 signalling ($p70^{S6K1\ T389}$ and $4E\text{-}BP1^{S65}$ phosphorylation) and a 1.9-fold increase in the rate of protein synthesis. Analysis of the global proteome detected an increase in peptides from 203 proteins and a decrease in peptides from 57 proteins. Of the proteins that were increased, gene ontology (GO) analysis revealed an overrepresentation in the biological process of 'translation' (i.e. protein synthesis; n=41 proteins), while the process of 'Mitochondrial ATP Synthesis Coupled Proton Transport' was overrepresented (n=7) in the proteins that decreased with FST. In addition, there was an upregulation of several components of the ubiquitin-conjugation and proteasome systems, including E2-ubuqitin conjugating enzymes, E3 ligases, proteasome subunits and DUBs. When normalised to changes in the total proteome, the ubiquitinomic analysis detected an increase in the ubiquitination of K residues on 34 peptides from 27 proteins, while there was decreased ubiquitination on 29 peptides from 24 proteins. Biological process GO analysis of the proteins with decreased ubiquitination showed an overrepresentation of proteins involved in 'Muscle Contraction', including myosin heavy chains. Furthermore, several ubiquitin-associated proteins also underwent changes in their ubiquitination status, including the E3 ligase, Trim55/MuRF2. Finally, changes in ubiquitin chain-linkage types were interrogated by Western blot, with FST-induced muscle growth being associated with reduced K48 ubiquitination and no change in M1 or K63 ubiquitination.
Combined, the results show that the skeletal muscle ubiquitinome is dynamically regulated, not only during muscle atrophy, but also during rapid muscle growth, and this is associated with altered expression and/or ubiquitination status of ubiquitin-related proteins and decreased ubiquitination of myosin heavy chains. These data suggest that ubiquitination may play an important role in the remodelling required to facilitate skeletal muscle growth.
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Regulating the powerhouse – removing mitochondrial bias and avoiding some of the pitfalls in exercise proteomics.

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The development of core facilities has led to proteomics becoming increasingly accessible to exercise physiologists interested in a deeper understanding of adaptations to exercise. However, while obtaining the data is becoming 'easier', probing this data demands careful considerations, spanning instrument variations, analysis software, and reagent availability. Other factors that wield significant influence over data quality and reproducibility, include data analysis and bioinformatics. While proteomics use within human skeletal muscle studies is still an emerging field, current hallmark studies, which pave the way for future research, contain key issues within their proteomics analysis that highlight the need for refining the analytical conventions used.

The normalisation of mitochondria, which play a pivotal role in energy production and hence a fundamental part of exercise science analysis, is one such issue. Traditionally, citrate synthase normalisation has been employed, but its limitations underscore the necessity for novel normalisation strategies. Another vital facet revolves around addressing missing data through effective imputation strategies. Missing data is a known issue within proteomics analysis, with label-free quantification being the most likely to introduce missing data. Missing data can come in the form of MCAR and MNAR potentially introducing bias and hampering downstream analyses. While imputation techniques can aid in assisting with removing missing values, there are many ways in which this can be achieved and using the correct imputation method, depending on the resulting data, is vital for ensuring data is robust. Finally, the challenge of managing proteomics data from small participant cohorts also warrants cautious statistical interpretation, urging receptivity to the evolving literature with the completion of larger-scale, and reproducibility studies.

Bioinformatic and computational analysis of proteomics data within exercise science is a multifaceted endeavour. The involvement of these specialities before projects are underway is key, with collaborations needed to develop robust and integrative methods for skeletal muscle analysis. Development of these analytical pipelines using proteomics data necessitates the need for careful consideration of key attributes, and less emphasis on final inferences and the number of proteins identified, with result reproducibility underscoring the path to robust results.

Muscle membranes talk

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Skeletal muscle is essential for posture and movement and thus the independent living of people in society. Beyond the use of muscle for contraction this organ provides, for example, heat to maintain the constant body temperature in mammals, including humans. The muscle of mammals has evolved to use the same set of membranes to regulate the very different processes in muscle that: (i) convert electrical signals to contraction, excitation-contraction coupling (EC coupling) (ii) heat generation in the resting muscle, also called thermogenesis; (iii) generate fatigue to prevent muscle damage; and (iv) signal following exercise to provoke muscle adaptation, among other processes. The set of membranes that regulate these processes are the plasma membrane, which is largely invaginated to form the transverse tubular (t-) system; the sarcoplasmic reticulum (SR) that forms the Ca^{2+} storage and Ca^{2+} regulating organelle; and the mitochondria that conducts resynthesis of ATP as metabolites are generated in the cytoplasm. These membranes exist in an intimate association with each other at defined locations across all sarcomeres of the fibre.

This talk will highlight the development of the mechanically skinned fibre preparation for fluorescence imaging on the confocal microscope. This preparation uniquely has its outer plasma membrane removed by microdissection with fine forceps to allow the trapping of Ca^{2+}-sensitive dye inside the t-system as these narrow tubules become seal at the former interface with the outer plasma membrane following mechanically skinning. This technique has been advanced to allow quantitative, high spatial and temporal detection of Ca^{2+} or H^{+} movements across the t-system membrane, ryanodine receptor (RyR) Ca^{2+} leak across the SR membrane, define the osmotic properties of the t-system membrane and provide structural information of this membrane network. Imaging dyes within the t-system have been combined with imaging Ca^{2+} in the SR or cytoplasm to characterise fundamental properties of EC coupling and store-operated Ca^{2+} entry (SOCE). Additionally, we have described how RyR Ca^{2+} leak in the resting muscle fibre is critical to setting the distribution of Ca^{2+} among the cytoplasm, SR, and mitochondria by setting the Ca^{2+} permeability of the t-system membrane. This mechanism is critical to regulating EC coupling, thermogenesis and signalling following exercise. Furthermore, this mechanism underlies pathology of malignant hyperthermia, RyR myopathies, some dystrophies and the decline in muscle function associated with ageing or poor lifestyle, which will be discussed.

Importantly, for translational as well as fundamental discovery projects, cut fibres obtained from needle biopsies of human muscle fibres can be used for confocal imaging of skinned fibres. Our approach has allowed cross-sectional and longitudinal studies of human muscle physiology and pathophysiology, which will also be highlighted in this talk.

Exercise induced fragmentation of the ryanodine receptor: A story of time, muscle fibre type, and fitness for physiological adaptations in skeletal muscle

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Introduction: The skeletal muscle ryanodine receptor (RyR1) channels are located in the membrane of the sarcoplasmic reticulum (SR) and are responsible for the controlled, passive release of Ca^{2+} from the SR into the cytoplasm, occurring during both the resting (i.e., RyR1 leak) and stimulated (i.e., contraction) states. As the primary regulator of Ca^{2+} storage, release, and uptake within skeletal muscle, the constant homeostasis of the SR compartments is paramount for muscle health and function. In the past decade, there has been increased interest investigating the adaptations of RyR1 channels and associated proteins in skeletal muscle in response to high-intensity exercise.

High-intensity interval training (HIIT) and sprint interval training (SIT) have become popular in today's society and are shown to be potent, time-saving form of exercises that improves mitochondrial content and function in human skeletal muscle (1, 2). Previous work has found that SIT causes extensive fragmentation of RyR1 protein channels of recreationally active human participants 24 hours following the exercise bout, causing prolonged force depression and Ca^{2+}-handling perturbations (3). Given the widely accepted knowledge that exercise-induced Ca^{2+} fluctuations stimulate downstream mitochondrial biogenesis signalling events (4), our aim was to investigate the time course of RyR1 protein channel fragmentation and the associated physiological adaptations as a result of SIT.

Methodology: All participants provided written, informed consent prior to enrolment in the study. The study was approved by the University of Calgary Conjoint Health Research Ethics Board (19-0423) and conformed to the standards set by the Declaration of Helsinki. Ten healthy, young females (n = 4) and males (n = 6) were recruited (age: 24 ± 4 years; height: 175 ± 8 cm; weight: 72 ± 9 kg; body fat percentage: 18 ± 7%; $\dot{V}O_{2max}$: 48.4 ± 7.7 ml/kg/min) to perform a session of SIT (6 × 30 s 'all-out' with 4.5 min rest after each sprint effort) with *vastus lateralis* muscle biopsy samples collected before and 3, 6, and 24 h after exercise. Whole muscle and pooled muscle fibre samples were analysed for relative protein content using low-volume Western blotting, and mRNA expression using quantitative real-time PCR.

Results and Conclusions: In the whole muscle homogenates, full-length RyR1 protein content was significantly reduced 6 and 24 h post-SIT ($p < 0.05$ for both) compared to pre-exercise. Peak full-length RyR1 protein reduction across the time course was examined in pooled fibres and found to significantly reduce in type II fibres ($p < 0.05$) but not type I fibres ($p < 0.05$). Three-hours post-SIT, there was also a reduction in fibre-type specific SR Ca^{2+} protein pumps, with a decrease in sarco-endoplasmic reticulum ATPase (SERCA) 1 in type fibres ($p < 0.05$) and SERCA2a in type I fibres ($p < 0.05$), in contrast to no time effect for either SERCA protein in whole muscle ($p > 0.05$). Peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC1α) mRNA expression was elevated 3 and 6 h post-SIT ($p < 0.05$ for both), but peak PGC1α was not significantly correlated with peak RyR1 fragmentation ($r^2 = 0.10$; $p > 0.05$). In summary, altered Ca^{2+}-handling protein content, which occurs primarily in type II muscle fibres, may influence signals for mitochondrial biogenesis as early as 3–6 h post-SIT in recreationally active humans.

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Maternal nutrition can rescue a nephron deficit in growth-restricted offspring.

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Low birth weight babies and premature babies are born with a deficit in nephron endowment, and an associated increased risk of hypertension and chronic kidney disease in later life. Strategies to boost or rescue nephron endowment are therefore likely to decrease the prevalence of these chronic conditions. We previously reported that mice fed a low carbohydrate/high fat diet (LCHFD) during pregnancy and postnatal life produced offspring with augmented nephron endowment. Here we examined whether this boost in nephron endowment was associated with an extension of the nephrogenic period, and whether a maternal LCHFD administered after birth could rescue nephron endowment in growth-restricted offspring born with a nephron deficit.

C57/Bl6J mice were fed either a normal diet (ND), low protein diet (LPD) or LCHFD throughout gestation and during postnatal life until postnatal day 21 (P21). Unlike humans in which nephrogenesis ends before term birth, nephrogenesis in mice continues for 3 days after birth, with most nephrons formed in this early postnatal period. Subsets of ND and LPD offspring were switched to the LCHFD at birth. Nephrogenic niches (SIX2 positive) and maturing glomeruli (PNA positive) were counted to assess in nephron formation at birth. To assess when nephrogenesis ceased, histological sections from P2 to P6 offspring were immunostained for SIX2 or LEF1 and the presence/absence of nephron progenitor cells and early committing nephrons determined. At P21, total nephron number per kidney was assessed using unbiased stereology.

Our results show that augmented nephron endowment in offspring from mothers on a LCHFD during pregnancy and lactation was associated with a 1-day extension of nephrogenesis, but was not associated with increased caloric intake. Compared with ND offspring, LPD offspring had a 20% nephron deficit at birth and a 30% deficit at the end of nephrogenesis. Switching the mothers of LPD offspring at birth to the LCHFD diet completely rescued the nephron deficit in offspring without extending nephrogenesis. This nephron rescue was associated with increased caloric intake.

Our studies demonstrate that a nephron deficit can be rapidly rescued during lactation by altering maternal nutrition. These findings suggest that nutrition may be able to boost nephron endowment in premature and growth-restricted babies and thereby reduce their risk of chronic diseases. The potential of food (including milk) composition during the final days of nephrogenesis to boost nephron endowment is of significant clinical relevance, especially for premature offspring. While the current study cannot delineate the impact of each macronutrient nor define the impact of the altered ratio of these macronutrients on nephron endowment, future studies to disentangle the impact of the three macronutrients on nephrogenesis are warranted.

Fetal growth restriction (FGR) alters cardiac metabolism in a sex- and cause-of-FGR-specific manner in the fetal guinea pig heart

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Introduction: Fetal growth restriction (FGR) due to maternal nutrient restriction (MNR) or maternal hypoxia (MH) predisposes offspring to an increased risk of cardiovascular disease in adulthood. In guinea pigs, we have previously shown that FGR due to MH or MNR decreases the expression of genes involved in fatty acid activation and transport into the mitochondria in the heart of offspring at 4 months of age, which was more prominent in males. To investigate whether these changes observed in the postnatal period are a result of *in utero* programming, the present study investigated the cardiometabolic profile of offspring *in utero.*

Methods: At ~30 days gestation, guinea pigs were allocated to control (21% oxygen, *ad libitum* food; *n*= 4F, 4M), MH (12% oxygen, *ad libitum* food but ate less than controls; *n*= 4F, 5M), and MNR (21% oxygen, 18-33% reduction in daily food intake per body weight of controls, daily ration matched to food intake per body weight of MH for equivalent gestational age; *n*= 5F, 5M). At ~65 days gestation (d; Term 69d) offspring were humanely killed (intramuscular injection of lethabarb, 200mg/kg) and LV+septum was snap frozen in liquid nitrogen for protein expression.1 male and 1 female pup whose body weight reflected the average litter were selected per dam for molecular analysis. Data was analysed using a 2-way ANOVA; $P \leq 0.05$ was considered significant.

Results: MH and MNR fetuses were asymmetrically growth restricted. MNR male, but not MNR females, had decreased cardiac protein abundance of oxidative phosphorylation (OXPHOS) complex I, II and IV compared to controls. In contrast, MNR females had increased complex V. Both MH male and female had decreased abundance of complex I, but only MH males had decreased abundance of complex II, IV and V. There was an overall effect of sex, with females having increased abundance of glucose transporter 4 compared to controls, irrespective of cause of FGR.

Conclusions: Our data suggests the postnatal phenotype observed in FGR offspring may be due to *in utero* programming. Specifically, the downregulation of OXPHOS complexes, dependent on sex and cause of FGR in these fetuses may indicate a reduced reliance on fatty acid oxidation that appears more prominent in males.

Facioscapulohumeral dystrophy (FSHD): Molecular mechanisms and therapeutic considerations

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FSHD is caused by the inappropriate expression of the embryonic transcription factor DUX4 in skeletal muscle. This distinguishes the pathophysiology of FSHD from most other muscular dystrophies and genetic diseases because the gene is not mutated but rather it is inappropriately expressed in skeletal muscle and retains its normal function. DUX4 is normally expressed at the onset of zygotic gene activation in the human 4 cell embryo and drives a portion of the first wave of embryonic gene transcription. DUX4 regulates the expression of factors that reprogram the transcriptome, epigenome, and proteome of the cells, establishing the totipotent state of the early embryonic cells. Mis-expression in skeletal muscle activates broad components of these early embryonic programs. Identifying the mis-expression of DUX4 as the cause of FSHD has also led to candidate therapeutics designed to suppress DUX4 expression or ameliorate the consequences. Clinical studies show that MRI and molecular biomarkers have the potential for measuring disease activity and progression. These studies also suggest a role of the immune system in both the early phases of the disease and possibly as a contributor to disease progression.

Extracellular Vesicles and their role in health and disease

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Extracellular vesicles (EVs) are small membrane bound particles released by cells into the extracellular space. The field of EV research has developed rapidly over the last decade from the study of fundamental biology to a subject of significant clinical and physiological relevance. They play roles in intercellular communication and in regulating a range of biological processes. EVs carry protein and genetic cargo, and as they are released during normal physiological conditions and pathological processes contain specific cargo related to the state of the cell from which they originate. The potential of harnessing EVs for the diagnosis or treatment of diseases such as cancer, neurological, and cardiovascular disorders is a current focus in the field. Accordingly, the applications of EVs as therapeutic targets, biomarkers, novel drug delivery agents and standalone therapeutics are being actively explored. This talk will provide an overview of the characteristics and physiological functions of the various classes of EV and highlight their potential of diagnostic use for diseases of the ageing brain.

Cardiac glycophagy is involved in physiological glycogen handling in the heart post-exercise

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Cardiac glycogen accumulation can be observed in response to cardiac metabolic stress, in both pathophysiological and physiological settings. Understanding the regulatory mechanisms underlying the glycogen response to exercise may highlight differences from pathological mechanisms in disease states.
The aim of this study was to track the time-course of the cardiac glycogen response to physiological metabolic stress (exercise) and examine glycogen regulatory mechanisms.
Cardiac tissues were collected from mice following either 8 weeks voluntary running-wheel, 1hr high intensity interval (treadmill; 0, 2, 4 and 16hrs post-exercise) or exhaustive exercise (treadmill; 0, 2, 4 and 16hrs post-exercise). Mice were euthanised via CO_2 inhalation or cervical dislocation for tissue collection. Glycogen was measured by amyloglucosidase assay and protein expression evaluated by immunoblot.
Cardiac glycogen content was positively correlated with running distance over 8 weeks. Following high intensity interval exercise, delayed cardiac glycogen accumulation was evident at 16hrs (1.8-fold). Following exhaustive exercise, cardiac glycogen elevation peaked at 2hrs (3.7-fold) and remained elevated at 16hrs (1.6-fold). In this model, initial glycogen synthase activation was evident, followed by inactivation at 2 and 4hrs post exercise. Glycogen recovery towards basal levels was not associated with upregulation of the cytosolic glycogen degradation enzyme, phosphorylase, and may be mediated by autophagic-lysosomal breakdown (glycophagy). Upregulation of the glycophagosome protein, GABARAPL1, was observed at 16hrs post-exercise.
This study provides evidence that glycogen synthase drives the initial cardiac glycogen response to physiological metabolic stress, but phosphorylase-mediated glycogen degradation appears not to be involved in glycogen recovery. Evidence suggests a role for glycophagic degradation of glycogen post-exercise and further research is warranted.

Acetylation as a fundamental regulator of contractile function

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Skeletal muscle contractile function (i.e. its ability to generate force) underlies functional independence, and is an important determinant of chronic disease risk and mortality. Whilst the post-translational modification (PTM) of specific contractile proteins through phosphorylation is important to contractile function in cardiac tissue, in skeletal muscle, other PTMs are thought to contribute. In recent years, reversible lysine acetylation, which is a PTM in which an acetyl group is added to a lysine residue, has been proposed to modulate numerous aspects of cellular biology, including metabolism, DNA damage and repair, cytoskeleton reorganization and endocytosis and vesicular trafficking, among many others. Interestingly, many proteins involved in excitation-contraction (E-C) coupling are also acetylated, although how this impacts their function, and subsequent effects on contractile function, are only beginning to be fully appreciated. The goal of this talk is to overview exciting new insights into the contribution of acetylation to skeletal muscle contractile function and physiology, and by extension, to emphasize the expanding importance of lysine acetylation to skeletal muscle biology.

Investigating the contribution of neuromuscular signaling in ALS/MND pathology.

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Background: Whether abnormalities in ALS/MND skeletal muscle contribute to the loss of nerve-muscle connections remains uncertain and settling this issue is important for developing effective treatments. The neural agrin-LRP4-Muscle Specific Kinase signalling system plays a vital role in the development of neuromuscular connections, and their maintenance throughout life. Neural agrin (***n*-agrin**) from the motor nerve acts via LRP4-Muscle Specific Kinase (**MuSK**) receptor complex on the muscle fiber surface to stabilise the neuromuscular synapse.

Objectives. Our goal is to better understand the molecular mechanisms that contribute to the loss of neuromuscular connections in ALS/MND.
Methods. We obtained muscle biopsies from 32 donors and used them to isolated muscle stem (satellite) cells. Muscle satellite cells were used to study their rate of myogenesis and for two different cell culture assays to test the ability of ALS/MND muscle cells to respond to pro-synaptic nerve signals. All participants provided informed consent. This study was approved by the relevant Ethics committees (HREC/13/QRBWH/58, HREC/14/QRBWH/495 and NHMRC/UQ ethics 20119003063 and 2015000022) and was conducted in accordance with the Declaration of Helsinki Principles.
Results. Satellite cells (resident muscle stem cells) isolated from ALS/MND muscle are slow to differentiate *in vitro*, which supports other reports of slower myogenesis in ALS/MND muscle. Importantly, muscle cells cultured from ALS/MND biopsies failed to respond to motor nerve terminal signals (human motor axons or n-agrin) to form the large clusters of AChRs that are essential for neuromuscular synaptic transmission. Moreover, the expression of *n*-agrin's receptor LRP4 and MuSK's downstream effector molecule Dok7 is lower in ALS/MND muscle cells.

Discussion. Our studies reveal that ALS/MND muscle fails to respond to either the presence of human motor axons or n-agrin. These findings suggest a fault within ALS/MND muscle due to either a defective synaptic agrin-MuSK signaling pathway, or a related fault in myosatellite myogenesis. To investigate the former, we are now attempting to restore ALS/MND muscle responses to n-agrin by increasing the expression of LRP4 and Dok7 using viral therapies.

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Unraveling a Neuroendocrine Ensemble Orchestrating Metabolic Adaptation in the Hypothalamus

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Energy deprivation triggers a profound metabolic shift orchestrated by intricate counterregulatory mechanisms. The hypothalamus emerges as a central node in coordinating the holistic response necessary to adapt metabolism to the body's current requirements. Traditional approaches to studying the hypothalamus have focused on categorising neurons based on peptide/receptor expression, limiting our ability to uncover their functional relationships. Consequently, the functional neuronal circuitry orchestrating metabolic adaptation remains largely uncharted.
To bridge this knowledge gap, we employed Targeted Recombination in Active Populations (TRAP) to functionally pinpoint the neuronal circuit governing metabolic adaptation. Our methodology unveiled a neuroendocrine ensemble situated in the mediobasal hypothalamus, pivotal in regulating whole-body metabolism. Single-cell transcriptomic analysis revealed the ensemble's inherent heterogeneity, encompassing both canonical metabolically relevant neurons and non-canonical neurons. Activation of this ensemble through selective chemogenetic methods resulted in rapid increases in body weight and adiposity, driven by elevated caloric intake, heightened food-seeking behaviour, reduced energy expenditure, and alterations in fuel utilisation. *In vivo,* metabolic tracing unveiled heightened fatty acid uptake and triglyceride incorporation in the liver, alongside a shift toward carbohydrate oxidation in muscle. Subsequent lipidomic analysis corroborated these tissue-specific nutrient shifts.
Notably, ablating this neuronal circuit using Caspase-3 or Diphtheria Toxin rendered the organism unable to stimulate feeding and suppress energy expenditure during periods of energy deficits, underscoring its essential role in orchestrating whole-body metabolic reprogramming. Additionally, by combining TRAP technology with CRISPR-Cas9 gene editing to selectively target insulin receptors within the ensemble, we identified postprandial pancreatic insulin secretion as a critical hormonal cue governing brain-body metabolic reprogramming. Disruption of insulin signalling to this ensemble induced a hyperactive state in these neurons, resulting in significant increases in body weight, adiposity, food intake, and reduced energy expenditure.
In summary, our findings spotlight a novel neuroendocrine ensemble at the forefront of metabolic adaptation in response to metabolic demands. This study not only sheds light on the critical role of neuronal ensembles but also establishes them as pivotal initiators of a complex array of physiological, behavioural, and biochemical processes central to metabolic homeostasis.

Does skeletal muscle stop ageing physiologically?

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Ageing is associated with an exponential increase in mortality, but paradoxically, in many organisms mortality rates decline late in life, a phenomenon known as late-life mortality deceleration. How latelife differs to ageing physiologically, and if mortality deceleration implies that ageing stops or reverses at a specific point of an organism's life remains unknown. Therefore, to examine the cellular and metabolic basis for mortality deceleration, we used a novel model of ageing – that of the African killifish, an extremely short-lived vertebrate that displays mortality deceleration. Using skeletal muscle, where the stereotypic hallmarks of ageing are well characterized, we highlight that ageing and late-life phases are physiologically distinct. Using a systems metabolomics approach, we demonstrate that during ageing there is a striking depletion of triglycerides, mimicking a state of calorie restriction, which triggers mitohormesis, a reactive oxygen species mediated stress resistance mechanism. This improves lipid and mitochondrial metabolism, subsequently maintaining nutrient homeostasis during late-life and driving mortality deceleration. Our results not only provide evidence of mitohormesis in regulating lifespan in vertebrates that naturally live-longer, but they also collectively show that the metabolic hallmarks of ageing are reversible.

Dysfunctional muscle Ca^{2+}-thermal signaling in malignant hyperthermia model mice

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Thermoregulation is one of the most important regulatory mechanisms in mammalian life. When this thermoregulatory function is impaired, the consequences are severe, including fever, heat stroke, and malignant hyperthermia (MH). What is the relationship between heat production and the biomolecular functions that cause this dysfunction of "thermal signaling"? The ryanodine receptor type 1 (RYR1) plays a key role in Ca^{2+} release from the sarcoplasmic reticulum (SR) during skeletal muscle excitation-contraction coupling. Genetic mutations in the *RYR1* gene are associated with MH. MH is a serious complication characterized by skeletal muscle rigidity and elevated body temperature in response to commonly used inhalational anesthetics. To date, more than 300 mutations in the *RYR1* gene have been reported in patients with MH. Recently, we generated an MH mouse model (R2509C-RYR1 mice) carrying a p.R2509C mutation in RYR1 using the CRISPR/Cas9 system. In R2509C-RyR1 heterozygous mice, MH-like episodes were induced by volatile anesthetics as well as by an increase in ambient temperature. We simultaneously measured intracellular Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) and cellular temperature, and found that an increase in cellular temperature is associated with an increase in $[Ca^{2+}]_{cyt}$ upon application of isoflurane. Furthermore, our progress extended to the successful expression of a genetically encoded Ca^{2+} indicator within skeletal muscles. This methods enabled us to monitor temperature changes and $[Ca^{2+}]_{cyt}$ alterations in real-time within living mice under conditions of MH.

Intestinal amino acid transporters as critical gatekeepers of nutrient homeostasis

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An imbalance of amino acid homeostasis occurs in several diseases. Elevated plasma levels of branched-chain amino acids (BCAA), for instance, are associated with Type II diabetes (T2D). High levels of phenylalanine occur in phenylketonuria (PKU), where patients lack functional phenylalanine hydroxylase (PAH), the first enzyme involved in the breakdown of phenylalanine. Therefore, restoring amino acid homeostasis could be a potential treatment strategy for these diseases.

The apical broad range of neutral amino acid transporter B^0AT1 (SLC6A19) is expressed in enterocytes of the small intestine and kidney proximal tubule epithelial cells. It mediates active absorption of all neutral amino acids, including phenylalanine and BCAA. B^0AT1 is a heteromeric membrane transporter, requiring the co-expression of angiotensin-converting enzyme 2 (ACE2) or collectrin in the small intestine and kidney, respectively, for trafficking, surface localisation, and catalytic function. Due to its role in amino acid homeostasis, B^0AT1 is a potential target to treat T2D and PKU because blocking its transport reduces the absorption of neutral amino acids in the intestine and causes spill over of neutral amino acids into the urine. Extensive target validation for T2D has been performed previously by us and by others for PKU.

We have developed a number of tools to identify and characterise inhibitors of human B^0AT1, including high-throughput assays, secondary screening assays, transgenic cell lines, which were used to identify potent inhibitors of the transporter. The potency of these inhibitors was further improved by medicinal chemistry. The ultimate aim is to generate specific inhibitors that block the transporter with nanomolar affinity and block both intestinal and renal neutral amino acid transport.

A super complex issue: are there sex-specific changes in mitochondrial respiratory supercomplex assembly following high-intensity interval training in humans?

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Background: Mitochondria are double membrane-bound organelles vital to many key biological processes, including energy generation via oxidative phosphorylation (OXPHOS). The mitochondria facilitate OXPHOS through the creation of a chemiosmotic gradient by four large multiprotein complexes (I-IV) of the electron transport chain. Although these complexes can exist freely in the inner mitochondrial membrane, they are often organised into large multicomplex structures called supercomplexes (SCs). The exact function of SCs is not clear, with theories including improved stability of individual complexes, improvement of substrate channelling, and reduction of deleterious reactive oxygen species production (1). Exercise has arisen as a promising non-pharmacological treatment for various metabolic diseases through its ability to improve mitochondrial characteristics in skeletal muscle, including the assembly of SCs. However, conflicting findings have been reported regarding the effects of training on SCs; one study using moderately active young men found no change in the proportion of SCs to mitochondrial content (2), while another in an elderly population reported an increase in the proportion of SCs relative to free complexes (3). Interestingly, while most exercise training studies have recruited men, there is emerging evidence of sex-specific mitochondrial adaptations to training (4). However, no study has investigated if there are sex-specific changes to SCs following training. Further assessment of the effects of exercise training on SC assembly is clearly warranted, particularly using cohorts of both men and women. **Aim:** This study aimed to identify sex-specific changes in mitochondrial respiratory supercomplex assembly in human skeletal muscle after 8 weeks of high-intensity interval training (HIIT). **Methods:** To assess SC composition, Blue Native PAGE (BN-PAGE) was performed on isolated mitochondrial extracts from skeletal muscle biopsies taken from 9 healthy untrained men (26.8 ± 5.7 y; 178.1 ± 9.6 cm; 78.9 ± 9.6 kg) and 9 healthy untrained women (28.7 ± 5.8 y; 166.3 ± 6.3 cm; 68.1 ± 8.5 kg) before and after 8 weeks of HIIT. Training consisted of four sessions per week, with each session consisting of 4 x 4-min intervals separated by 2 min of rest. The intensity of each interval was set between each participant's lactate threshold (W_{LT}) and peak power output (W_{peak}), and corresponded to approximately 80% of each participant's VO_{2peak}. To measure changes in mitochondrial respiratory function following training, permeabilised muscle fibres were analysed in an Oxygraph-2k high-resolution respirometer. To assess changes in mitochondrial content following training, citrate synthase activity of homogenised muscle lysate was measured. **Results:** Increases in SC protein content were observed for both men ($p<0.05$) and women ($p<0.05$) following 8 weeks of HIIT; however, these changes were proportional to individual complex protein content and mitochondrial content ($p>0.05$). Additionally, no correlation between changes in SC protein content and mitochondrial respiratory function (as assessed with high-resolution mitochondrial respiration) was detected. No effect of sex was observed for any changes in free complex or SC assembly following training, despite a larger increase in VO_{2peak} observed for men than women ($p<0.05$). **Conclusion:** Our results indicate mitochondrial respiratory supercomplex assembly increases proportionally to mitochondrial content in both men and women, adding new evidence for a non-energetic role of supercomplex structures that is independent of sex.

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Serial notexin injury in wild-type mice produces functionally normal regenerated skeletal muscle with increased fibre branching that's protected from eccentric injury

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Background:
Skeletal muscle possesses the remarkable ability to regenerate after damage caused by various insults, including physical trauma, chemical injury, or genetic diseases such as muscular dystrophy. This regenerative process results in the formation of muscle fibres with centrally located myonuclei and often leads to the development of branched fibres. While extensive research has focused on muscle regeneration and its connection to diseases like muscular dystrophy, the impact of branched fibres on the functional properties of healthy regenerated muscle remains largely unexplored.
Methods:
In this study, we isolated fast-twitch extensor digitorum longus (EDL) and tibialis anterior (TA) muscles from wild-type (WT) mice subjected to notexin induced muscle injury. For the injection procedure (approved by MCRI AEC), 40uL of notexin (0.2ug; Latoxan) was injected longitudinally into the right EDL outwards towards the distal tendon whilst the mouse was under anaesthetic (isoflurane 2% via nose cone). Muscles (TA and EDL) were collected either 21 days after a single Notexin injection (1X Notexin) or following three consecutive Notexin injections (3X Notexin) and compared to undamaged (control) muscles. We assessed contractile force, fatigue and susceptibility to eccentric muscle injury, as well as histology, the presence of branched fibres and differences in gene expression. Statistical analysis was performed using ANOVA.
Results:
Muscles subjected to 3X Notexin regeneration displayed remarkable differences compared to control and 1X Notexin muscles. They exhibited significant hypertrophy, with increased muscle mass and cross-sectional area. Notably, 3X Notexin-regenerated muscles were highly resistant to eccentric contraction-induced damage, recovering almost 100% of their maximum force 60 minutes after the protocol. This protective effect correlated with a substantial increase in both the number (~70%) and complexity (~22%) of branched fibres in 3X Notexin muscles compared to 1X Notexin and control muscles. Importantly, the contractile properties, twitch kinetics, and force-frequency characteristics of 3X Notexin-regenerated muscles were similar to those of controls, indicating the preservation of muscle function despite increased fibre branching.
Conclusions:
Repeated regeneration of WT mouse fast-twitch EDL muscles through 3X Notexin injections results in muscle hypertrophy and a significant increase in the number and complexity of branched fibres. This branching appears to protect the regenerated muscle from eccentric contraction-induced damage by effectively increasing the number of shorter muscle fibres in series, contributing to muscle stiffness and resilience. These findings shed light on the functional implications of branched fibres in regenerated muscle and may have implications for understanding and potentially enhancing muscle regeneration in various pathological conditions.

Cardiac trabecular fate is genetically hardwired prior to sprouting morphogenesis

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One of the processes which increases myocardial mass is that of trabeculation – the formation of myocardial protrusions into the lumen of the heart. In zebrafish, trabeculation occurs when cardiomyocytes from the "compact" single layer myocardium undergo partial delamination to seed the trabecular layer. The earliest known mechanism in this process is mechanical tension, which is increased in select cardiomyocytes driving delamination from ~60 hours post fertilisation (hpf).
We have generated a transgenic line (*Tg(cbfa2t3:gal4;uas:gfp)*) which shows expression in individual cardiomyocytes as early as 40 hpf – ~20 hours prior to delamination. The transgene is expressed in the single-layer compact myocardium, and by 6 days post fertilisation (dpf), all trabecular cardiomyocytes express the transgene, leaving transgen1e-negative cardiomyocytes in the compact wall. Lineage tracing through photoconversion and Brainbow labelling show the majority (>90%) of transgene-positive cardiomyocytes end up in the trabecular layer, suggesting the transgene is a marker of trabecular fate. Chemical treatment and genetic manipulations show that the expression of *Tg(cbfa2t3:gal4;uas:gfp*) is independent of Nrg/ErbB signalling, a key driver in trabecular growth. Other factors known to regulate trabeculation, including haemodynamic forces, endocardial signalling and mechanical tension, either mildly reduce or don't impact the number of *Tg(cbfa2t3:gal4;uas:gfp*)-positive cardiomyocytes, suggesting trabecular fate is regulated by mechanisms distinct from trabecular delamination and growth.
Based on these data, we propose that fate specification is a new and previously unappreciated step in trabecular development. This identity is genetically hardwired early in cardiac development and is temporally and genetically separable from existing known mechanisms that regulate trabeculation.

Upgrading Metabolic Disease Treatment by Reversing Glucagon-Like Peptide-1 Resistance in the Brain

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Incretin-based therapeutics, exemplified by glucagon-like peptide-1 receptor agonists (GLP-1RAs), represent the forefront of treatments for obesity and Type-2 diabetes. However, a growing concern revolves around their limited effectiveness in severely obese patients (BMI>40), where over 20% fail to achieve even a modest 5% reduction in body weight[1]. This challenge underscores the need to address potential resistance to incretin peptides, particularly in advanced metabolic disease states.

Recent evidence suggests that GLP-1RAs primarily act upon GLP1R expressed within the brain to attenuate feeding behaviour and improve glycaemic control. Exactly how or where GLP-1RAs act in the brain is unclear, and it is entirely unexplored whether this signalling goes awry in obesity/T2D. In this study, we unveil a critical impediment to GLP-1RAs' efficacy within the arcuate nucleus of the hypothalamus (ARC) in pre-clinical models of metabolic disease. We pioneered a euglycaemic GLP-1RA clamp to assess whole-body GLP-1RA sensitivity and identified that obesity/T2D are associated with whole-body insensitivity to GLP-1RA. This GLP-1RA insensitivity is recapitulated following CRISPR-mediated ablation of *Glp1r* within the ARC of lean adult mice.

To mechanistically explore the obesity-impaired GLP-1RA signalling within the ARC, we systemically administered a fluorescently labelled GLP-1RA and assessed its real-time biodistribution within the ARC *in vivo* within lean and obese/T2D mice. We reveal a specific reduction in GLP-1RA extravasation from the blood into the ARC parenchyma in obesity/T2D, suggesting impaired access and signalling of GLP-1RA into the ARC in metabolic disease.

Recent evidence has identified a chondroitin-sulphate proteoglycan extracellular matrix (CSPG-ECM) that surrounds ARC neurons and regulates bloodborne hormone extravasation. We identify the CSPG-ECM surrounding GLP1R expressing ARC neurons becomes remodelled and augmented in obesity/T2D. We further identify that GLP1-RAs directly interact with core proteoglycan components of the CSPG-ECM potentially sequestering drug/receptor interactions. Collectively this suggests a direct functional relationship between GLP1-RA signalling and the CSPG-ECM in the ARC. To confirm this, we enzymatically ablated the CSPG-ECM in the ARC of obese/T2D mice which restored GLP-1RA biodistribution and signalling within the ARC resulting in an enhancement of GLP1-RA actions upon the remission of obesity and T2D.

Our study identifies GLP-1 resistance within the brain in metabolic disease states. Attenuating the CSPG-ECM surrounding ARC neurons improves GLP-1RA resistance enhancing its whole-body efficacy in treating metabolic disease. This research offers an avenue for improving GLP-1RA's actions to promote weight loss and improve glycaemic control in obesity/T2D.

*All surgeries were performed under isoflurane anaesthetic administered via inhalation through a nose cone.

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Metabolomic adaptations to prolonged bed rest in energy balance are linked to fuel utilisation and glucose disposal

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Long term bed rest is linked with a rapid decline in glucose disposal and the development of metabolic inflexibility, however little is known about the mechanisms underlying such changes. Mechanistic studies may be confounded by failure to control energy intake which leaves individuals with a net positive energy balance and thus the accumulation of intramyocellular lipid content. Previously it was shown that when young, healthy males were maintained in energy balance during bed rest, insulin mediated glucose disposal and increases in carbohydrate oxidation were reduced while the inhibition of lipid oxidation was blunted despite no increase in intramyocellular lipid accumulation (1). Metabolites represent the endpoints of regulatory networks and provide a chemical link between changes occurring in genomic and phenomic level. Adaptations in the metabolome during an intervention therefore reflect any observed physiological adaptations occurring in response to an internal or external stimulus which disrupts the normal biological state. Moreover, undertaking a systems biology approach by adopting metabolomics techniques in traditional intervention studies can provide greater insight into complex physiological mechanisms. Here, we adopted an untargeted metabolomics approach to profile the plasma metabolome and investigate the mechanisms underlying the changes in glucose disposal and substrate oxidation.
Healthy males (n=20, 34±1.8yrs) underwent 56 days bed rest while in energy balance. Plasma was collected 6 days prior to starting bed rest and on day 56 of bed rest. Measurements of glucose disposal, fat and carbohydrate oxidation, acquired under a hyperinsulinaemic euglycaemic clamp, and body composition, acquired by dual-energy X-ray absorptiometry, were also collected before and after bed rest. Untargeted analysis was performed using UHPLC-MS/MS to detect all possible plasma metabolites before and after bed rest. Partial least squares discriminant analysis was used to classify pre- and post-bed rest samples. Pathway and network analysis of significant metabolites was used to identify mechanisms underlying metabolic changes.
Of the 16,462 plasma metabolites detected, 2835 metabolites were found to be at a significantly different abundance after bed rest. Putative identification of metabolites identified the predominant response to bed rest was dysregulation of whole-body substrate oxidation, with several enriched pathways linked to lipid oxidation. Furthermore, associations between the global plasma metabolome and endpoint measures of change in glucose disposal, rate of carbohydrate oxidation and rate of fat oxidation were assessed by weighted correlation network analysis which found robust correlations between endpoint measures and metabolite expression, suggesting metabolomic disturbances are closely linked with functional outcomes in deconditioning.

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Exercise-responsive gene transcription is differentially affected by an interleukin-6 promotor variant in male and female mice

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Background: Interleukin-6 (IL-6) is a pleiotropic cytokine that is secreted from skeletal muscle during exercise. Acute increases in IL-6 signalling are important for coordinating metabolic benefits from exercise training. A common (up to 40% prevalence in New Zealand) genetic variant single nucleotide polymorphism in the IL-6 promoter region, rs1800795 (-174 G>C) is located in close proximity to the activator protein-1 and cyclic AMP-response element transcription factor binding sites that are known to mediate IL-6 transcriptional control during exercise. However, whether the IL-6 variant alters the transcriptional response to exercise has not been previously investigated.
Methods: Knock-in mice (both sexes) were generated with IL-6 GG wild type or CC variant genotype for rs1800795. Peak running speed was determined using a VO_2Max treadmill ramping to exhaustion protocol. Mice then performed 60 minutes of high intensity interval treadmill running (HIIT) alternating between 2 minutes at 50% and 90% of the peak running speeds. Mice were euthanized with CO_2 at rest, immediately and at 4 hours post 60 minutes HIIT. Blood samples were then collected via cardiac puncture and tissues were collected and snap frozen in liquid nitrogen. IL-6 levels in blood plasma were quantified using ELISA and mRNA expression of target genes were quantified using qPCR.
Results: Despite both genotypes having similar peak maximal running speed, immediately after 60 minutes of HIIT, both sexes of the variant CC mice exhibited a ~2-fold greater increase in skeletal muscle IL-6 mRNA and circulating IL-6 compared with wild-type GG mice. At 4 hours post exercise, male variant CC mice displayed downregulated transcription of genes controlling glucose metabolism and transport (~2-fold lower PPAR-γ and GLUT4 mRNA vs wild-type GG mice). PGC1-α displayed a similar exercise-induced increase in expression in both GG and CC mice. In female variant CC mice, exercise-induced PGC1-α mRNA expression was augmented relative to wild-type GG mice. PPAR-y and GLUT4 mRNA were unchanged. Genes controlling mitochondrial biogenesis were upregulated with exercise in CC vs GG mice of both sexes (~2-fold higher TFAM and ~3-fold higher NRF1 mRNA).
Conclusion: This study provides the first evidence that exercise-induced increased IL-6 production in mice with the IL-6 variant (rs1800795) is associated with alterations in exercise-responsive gene transcription. These findings suggest that, for people with the IL-6 variant, transcriptional differences in genes involved in regulating metabolism may alter the metabolic health benefits gained from exercise training regimens. Further investigation into the role of the IL-6 promoter variant in modulating substrate utilisation during exercise is now warranted.

Empagliflozin may positively affect advanced dystrophic muscle

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Background: Fat infiltration, oxidative stress and mitochondrial dysfunction is evident in Duchenne muscular dystrophy (DMD). Empagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor used in the treatment of type 2 diabetes mellitus to prevent glucose reabsorption and stabilise glycaemia. However, other suggested effects of empagliflozin, including reduction in fat content and improved mitochondrial function, could prove to be of benefit to dystrophic muscle. Thus, the aim was to investigate whether there is potential for empagliflozin to be repurposed for the treatment of DMD.
Methods: Aged C57BL/10 *mdx* mice and wildtype C57BL/10 mice (~19 months old) mice were fed a custom-made diet with or without empagliflozin (300 mg/kg of food) and were randomly allocated into four groups: wildtype control diet (n=12), wildtype empagliflozin diet (n=8), *mdx* control diet (n=6), or *mdx* empagliflozin diet (n=6). After 4 weeks of treatment, resting blood glucose, grip strength and body composition were assessed. Animals were then anaesthetised with isoflurane (4% induction, 2% maintenance) and extensor digitorum longus (EDL), soleus and diaphragm muscles were dissected for *ex vivo* contractile function, while the flexor digitorum brevis (FDB) was extracted for mitochondrial analysis, and the tibialis anterior (TA) processed for histological analysis. All experiments were approved by the Victoria University Animal Ethics committee (AEC 22/005).
Results: As expected, empagliflozin decreased resting blood glucose ($p<0.0001$) in both wildtype and *mdx* mice. Mitochondrial uncoupling efficiency was also reduced ($p=0.0196$) in both strains with treatment. Although *mdx* mice had lower lean mass index ($p=0.0113$) compared to wildtype, treatment was unable to induce any changes in fat or lean mass. However, empagliflozin was able to improve grip strength ($p=0.0367$) in *mdx* mice. Empagliflozin was also able to significantly improve absolute and specific muscle force in *mdx* diaphragm ($p=0.0192$ and $p=0.005$, respectively), as well as force development at 60Hz in *mdx* EDL ($p=0.0345$). Additionally, empagliflozin lowered fat content in wildtype mice TA muscle ($p=0.0062$) and reduced the number of small regenerating fibres in *mdx* TA muscle compared to their untreated counterparts.
Conclusion: Empagliflozin improved resting blood glucose and lowered uncoupling efficiency, suggesting a reduced susceptibility to oxidative damage in both wildtype and *mdx* mice. In *mdx* mice, empagliflozin also reduced the number of small regenerating fibres, suggesting a reduction in damage and completion of the regeneration process. Combined with muscle improvements, including increases in diaphragmatic muscle force and increased force development at 60Hz in the EDL, these data support the need for further work to investigate the capacity of empagliflozin to positively affect dystrophic muscle at earlier stages of life.

Importance of vitamin D for limiting immobilisation-induced muscle loss

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Introduction: Loss of skeletal muscle mass occurs secondary to chronic disease and can be caused by many catabolic stimuli. However, muscle loss also occurs acutely, as with bed rest or limb immobilisation, with lower muscle mass increasing length of stay in hospital. Given low muscle mass conditions such as sarcopenia increase likelihood of adverse events, as well as being associated with higher morbidity and mortality, investigating ways to limit muscle loss is vital. Vitamin D (vitD) is well known for its importance in the regulation of calcium and bone mass and given the presence of the vitD receptor in skeletal muscle, it is presumed that vitD also regulates muscle mass. Indeed, people deficient in vitD often present with muscle weakness and fatigue, with supplementation effective in reversing these symptoms. However, whether vitD directly effects muscle mass and function, particularly in a setting of acute, rapid muscle loss, is unknown. Thus, this project manipulated levels of vitD and investigated the loss of muscle in a pre-clinical mouse model of bed rest/inactivity commonly observed in hospital admissions.
Methods: 8-week-old male C57Bl/10 mice (n=12 per group) were fed for 6 weeks on a diet containing normal (1000 IU/kg; NORM), low (0 IU/kg; LVD), or high (20,000 IU/kg; HVD) cholecalciferol (inactive bioavailable vitD) content. After 4 weeks of feeding, animals were anaesthetised with isoflurane (4% induction, 1-2% maintenance) and casts were applied to the right hindlimb of each mouse to induce atrophy. Following 2 weeks of casting, animals were again anaesthetised with isoflurane, casts were removed and the extensor digitorum longus (EDL) and soleus (SOL) muscles extracted for contractile function and fatigue analysis and tibialis anterior (TA) muscles extracted for histological analysis. Animal experimentation was approved by Victoria University Animal Ethics Committee (AEETH 22/005).
Results: Immobilisation induced atrophy in EDL, SOL and TA muscles ($p<0.0001$), demonstrated by reduced muscle mass (EDL & SOL) and reduced cross sectional area (TA) compared to the uncasted muscles. Absolute force was decreased in the immobilised EDL muscles in both the LVD ($p<0.0001$) and HVD ($p<0.001$) diet groups, but not the NORM diet. LVD immobilised EDL force was lower than both the immobilised HVD ($p<0.001$) and NORM diets ($p<0.001$). In the SOL, only the immobilised LVD was lower than the uncasted muscles ($p<0.01$). In the EDL, both the NORM ($p<0.0001$) and HVD ($p<0.01$) diet animals exhibited a significant leftward shift in the force frequency curve following immobilisation, which was further exacerbated in the LVD immobilised muscles ($p<0.0001$). No such effect was observed in the SOL muscles. A similar effect was seen in the fatigability of the EDL immobilised muscles, with a significant ($p<0.0001$) rightward shift in the NORM and HVD groups, and a further right shift in the LVD, but no effect in the SOL muscles. Histological analysis of TA muscles revealed a significant increase in percentage of unhealthy tissue in LVD immobilised muscles ($p<0.01$).
Discussion: The results exhibited by the EDL and TA in response to vitD modification and immobilisation suggests alterations to calcium handling and/or muscle fibre types. SOL muscles also atrophied in response to immobilisation but were not meaningfully impacted by vitD status. Immobilisation and LVD diet profoundly affected morphometric and functional characteristics of EDL muscles. Greater atrophy in a low vitamin D environment has implications for muscle loss with acute hospitalisation and bed rest. VitD depletion may act as a catabolic catalyst under conditions of skeletal muscle immobilisation, and more research is warranted to uncover mechanistic changes driving these results.

Exploring the role of human milk lipids in protecting the infant against non-communicable diseases

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Compared to formula-fed infants, breastfed infants have lower risk of obesity, diabetes, heart disease, and other non-communicable diseases. The mechanisms behind this protection, however, remain unknown. Human milk contains a complex lipidome, which may have critical roles in infant health and disease risk. Lipidomics analyses in early life are limited, and human milk lipidomics is challenging, thus research is still required to understand the role of lipids in early life. This study aimed to address key knowledge gaps in the human milk lipidome and explore possible implications for early life health.

We utilised four liquid chromatography-mass spectrometry methods (lipidome, triacylglycerol, total fatty acid, alkylglycerol) to analyse human milk samples from two birth cohorts, the Barwon Infant study (n=312) and University of Western Australia cohort (n=342). For comparison, bovine, goat, and soy-based infant formula, and bovine and goat milk, were also analysed. Data were explored as concentrations, relative abundance, and infant lipid intake. Statistical analyses included principal component analysis, mixed effects modelling, and correlation, with false discovery rate correction, to explore human milk lipidome longitudinal trends and inter and intra-individual variation, differences between sample types, infant lipid intakes, correlations between infant plasma and human milk lipids, and relationships with other infant outcomes.

We identified 979 lipids with our lipidomics analyses. There were distinct differences between the human milk lipidome and that of infant formula and animal milk. Ether lipids were of particular interest due to their significantly higher concentration and relative abundance in human milk compared to formula and animal milk, if present in the latter samples at all. Many ether lipids were highest in colostrum, and many changed significantly throughout lactation. Furthermore, significant correlations were identified between human milk and infant circulating lipids (40% of which were ether lipids), and specific ether lipid intake by exclusively breastfed infants was 200-fold higher than that of an exclusively formula-fed infant. Our findings have potential implications for early life health, which we continue to explore, and may reveal why breast and formula-fed infants are not afforded the same protections.

The dichotomy of bariatric Surgery: Investigation of the metabolic benefits and adverse behavioral outcomes - insights from an animal model

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Background: Bariatric surgery has transformed the care of obesity. In addition to the well-documented effect on morbid obesity, bariatric surgery also results in significant improvements in many obesity-related comorbidities. Procedures such as the vertical sleeve gastrectomy (VSG) are valuable tools in modulating cardiac risk factors, particularly among severe heart failure patients who are contraindicated for heart transplantation. In such cases, VSG acts as a crucial intermediary, paving the way for definitive heart therapies. Importantly, patients demonstrate vast improvements in cardiac structural and functional parameters and in individuals with obesity and established heart failure, there is a reduction in major adverse cardiovascular events (MACE). In a short period of time, the focus of bariatric surgery outcomes has shifted from being exclusively related to weight loss (and prevention of cardiovascular disease through risk factor modulation), to now, being a potential therapeutic intervention for the management of heart failure. What is lacking in this recent shift to adopt dramatic and irreversible surgeries for the treatment of obesity-related heart failure is a detailed understanding of the cardiac structural and functional changes after bariatric surgery and the specific mechanisms that underpin the relationship of surgery to improvements in cardiac-related outcomes. We have recently demonstrated the weight loss and glucoregulatory effects are, at least partly, mediated by the rapid increase in energy expenditure in brown adipose tissue (BAT). Most recently, the metabolic benefits of active BAT have been extended to include cardiometabolic health, where individuals with active BAT had a lower prevalence of cardiometabolic diseases including heart failure. Therefore, in this study we aim to define the specific cardiac structural and functional changes after bariatric surgery and to determine the relationship of these changes to the recruitment of energy-burning brown fat, given its recently documented cardioprotective benefits.

Approach and Results: To simulate obesity-related heart failure, these studies utilise a concomitant metabolic and hypertensive stress model elicited by the combination of high-fat diet (HFD; beginning at 6 weeks) and infusion of Angiotensin II (10 weeks post HFD initiation) for two weeks via osmotic minipump - this model induces obesity and hypertension coupled with diastolic dysfunction and concentric hypertrophy with fibrosis. To evaluate the impact of VSG on cardiac morphology and function, cardiometabolic profiling will be performed over the first two weeks following VSG surgery which includes the measurement of body weight and food intake, the continuous measurement of BAT activity, in vivo cardiac function using echocardiography and post-mortem histological assessment of BAT and cardiac tissue.

Findings from the first series of studies in our mouse model of VSG highlight a rapid reduction in body weight that coincides with increased BAT activity and both structural (reduced left ventricular wall thickness) and functional (improved diastolic dysfunction, reduced IVRT) improvements in cardiac parameters. These studies are ongoing, and analysis will specifically focus on defining the histological and molecular changes than unpin our observations in vivo.

Conclusion: These studies pave the way for future mechanistic studies to better understand the nature of BAT – heart crosstalk to ultimately lead to the strategies that will recapitulate the cardiometabolic benefits of bariatric surgery with less invasive approaches.

Physiological determinants of extreme endurance exercise performance: lessons from the first sub-2 hour marathon

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The physiological determinants of distance running performance are widely accepted to be the maximal oxygen uptake (VO2max), the lactate threshold (LT), and running economy (RE). These three variables can be measured during an incremental treadmill test in the laboratory and used to predict race performances. However, it is rarely acknowledged that VO_2max, LT and RE are not static but rather are *dynamic* variables that change with time as fatigue develops during endurance exercise. Studies show, for example, that RE deteriorates and the critical speed (the highest speed that can be sustained in a steady-state) falls during 2 hours of heavy exercise. This has given rise to the notion of a '4^{th} dimension' in the physiology of distance running, namely, fatigue resistance or *resilience*. There is substantial inter-individual variability in the magnitude of resilience – and yet its physiological determinants are obscure. Moreover, the interaction between fatigue development, biomechanical alterations and changes in O_2 cost are under-explored such that causes and consequences are unknown. This lecture will explore this concept and consider whether the all-time greats of marathon running, including Paula Radcliffe and Eliud Kipchoge, achieve their greatness at least in part as a consequence of superior resilience. The lecture will also consider whether training, footwear or nutritional interventions might influence resilience and therefore enhance performance.

TGF-β1 induces metastatic colonisation to skeletal muscle in mouse models of metastatic breast cancer

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Cancer metastasis occurs by tumour cell dissemination throughout the bloodstream and lymphatics to distant organs. Selectivity exists between different tissues and cancers that dictates where metastases will develop. Common sites of metastasis include the lymph nodes, lung, liver, bone and brain. Intriguingly, skeletal muscles are rarely the site of secondary tumour growth despite making up 30-40% of a human's body mass and receiving a rich blood supply. The mechanism underlying why skeletal muscle has an apparent resistance to metastasis remain unclear.

This research aims to understand why muscles are infrequently affected by metastatic cancers. Based on previous studies showing TGF-β as a regulator of cancer cell dissemination and colonisation, we hypothesised that manipulation of TGF-β in muscle would render it more susceptible to metastasis.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). To test our hypothesis we increased expression of TGF-β1 through gene delivery locally in mouse muscles with concurrent orthotopic inoculation of mCherry-labelled 4T1.2 breast cancer cells while mice were anaesthatised under 3-5% isoflurane. Mice harbouring lung metastases were humanely killed with sodium pentobarbitone (60mg/kg) and muscles were assessed for evidence of cancer cells by histology, qPCR and flow cytometry.

Histological analysis and qPCR identified mCherry-positive tumour cells in TGF-β expressing but not control muscles. Intriguingly, this phenotype was associated with a 49.5% loss in muscle mass in these tumour-bearing muscles, with no difference seen in tumour-free controls ($p<0.05$). Colonisation of skeletal muscle occurred early in the progression of disease with tumour cells detected in muscle at the time of primary tumour excision. Furthermore, we found that colonisation was associated with an increase in intramuscular immune cells, and that skeletal muscle colonisation occurred to a lesser extent in immunocompromised mice.

These results demonstrate that altering the muscle microenvironment through TGF-β1 promotes colonisation and growth of cancer cells. Successfully defining the unique factors within muscles that deter metastatic propagation may identify potential anti-metastatic agents that could prevent metastases in vulnerable organs.

Extracellular vesicles are enriched in activated AMPK following acute exercise

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Background: Exercise is conceivably the most potent physiological activator of the fuel-sensing enzyme, AMP-activated protein kinase (AMPK), in many tissues in mammals. Extracellular vesicles (EVs) are significant carriers of biologically active cargo that traffic to local or distant organs or cells. We previously demonstrated that EVs containing proteins are released into the bloodstream during exercise and relocate to the liver (Whitham et al., 2018), where they can modify biological processes. Here we aim to understand how exercise may be affecting AMPK activity via EV related mechanisms.
Methods: In order to define potential mechanisms for these actions, we collected blood from both mice and humans, following exercise, and isolated EVs using ultracentrifugation (UC) and size-exclusion chromatography (SEC). Mice performed treadmill running for 1 hour at a speed of 10 meters/min for 10 minutes, before increasing by 2 meters/min every 10 minutes. Humans performed cycling for 1 hour at 50% VO_2max for 30 minutes 70% VO_2 max for 20 minutes and 80% VO_2max for 10 minutes.
Results: The presence of EVs was confirmed using nanoparticle tracking analysis, in SEC-derived mouse and human EV-rich fractions in both sedentary and exercise EVs and absence in the EV-poor fractions. Presence of EVs was also confirmed in UC-derived mouse and human EV-rich fractions and absent in EV-poor fractions. Presence of EVs was also confirmed via western blotting of classical EV markers CD9 and syntenin-1 in mouse and human EV-rich fractions compared with EV-poor fractions. We detected AMPK isoforms, α1, β1, β2, γ1 and γ2 in human-derived small EVs in mass spectrometry-based proteomics. We then examined AMP-activated kinase activity in both sedentary and exercise samples from mice and humans. In both species, exercise-derived EV-rich fractions following sonication, induced a significantly large increase in kinase activity compared with sedentary EV-rich fractions. Importantly, conducting the activity assay in non-sonicated EV-rich fractions from mice and humans, showed reduced kinase activity implying disruption of the phospholipid membrane on the EV is necessary.
Conclusion: Exercise-derived EVs irrespective of isolation method or species resulted in increased kinase activity compared with sedentary-derived EVs. We hope to further delineate how AMPK is trafficked into EVs and other potential kinases that may be responsible for this exercise-derived increase in kinase activity.

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Investigating skeletal muscle dysfunction in a mouse model of critical illness

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Intensive care unit-acquired weakness (ICU-AW) is a common neuromuscular complication associated with patients in the ICU, especially after sepsis, which increases patient mortality and contributes to health deficits post-discharge (Wang *et al*., 2020). Treatments remain elusive because of difficulties in conducting large, robust clinical trials in the ICU, difficulty in obtaining muscle biopsies, and inter-patient variability, and even greater barriers in conducting long-term follow up studies because of high rates of attrition post-hospital discharge (Wilcox & Ely, 2019).

Small animal models of critical illness can help better understand the mechanisms underlying ICU-AW and evaluate therapeutic strategies. The zymosan model of peritonitis involves the intraperitoneal (*i.p.*) administration of the endotoxin zymosan (Rooyackers *et al.,* 1994) a polysaccharide extracted from the cell wall of *Saccharomyces cerevisiae*, which causes a systemic inflammatory response through TLR-2 activation (Sato *et al.,* 2003). Unlike most animal models of peritonitis that are terminal, the zymosan model involves recovery, providing an opportunity for greater mechanistic insight into the long-term skeletal muscle deficits experienced by ICU-AW patients. In this study, we characterised the response of the muscles of the hindlimb and diaphragm to a septic insult in order to gain greater mechanistic insight into the morbidity associated with critical illness.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). C57BL/6J (16-week-old) male mice received an *i.p.* injection of zymosan (30 mg/100 g) suspended in liquid paraffin, to induce systemic inflammation, or an equivalent volume of liquid paraffin (n=5 per group). Paraffin vehicle control mice were pair-fed in accordance with zymosan treated mice to account for possible alterations in food intake. Mice were deeply anaesthetised using sodium pentobarbitone and killed via cardiac puncture 14-days post-zymosan injection, with terminal collection of blood and tissue for biochemical and histological analyses.

After the administration of zymosan, mice had reduced food intake ($P<0.001$) and body weight at day-one ($P<0.05$) and experienced a delayed recovery in body weight compared to vehicle treated mice. Despite apparent recovery from peritonitis, 14-days after the administration of zymosan, mice exhibited signs of systemic infection, including increased spleen ($P<0.01$) and liver ($P<0.01$) mass. Assessment of diaphragm muscle cross-sections from zymosan treated mice revealed smaller myofibres ($P<0.05$) and an increased number of CD68-positive fibres, indicating inflammation ($P<0.01$), compared with diaphragm muscles of vehicle treated mice. These findings are consistent with the diaphragm dysfunction often associated with critically ill patients in the ICU.

The Zymosan mouse model of critical illness has the potential to provide important insights into the mechanisms underlying ICU-AW and be a suitable experimental platform for evaluating treatments to attenuate sepsis and enhance the recovery of muscle function.

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Intestinal ceramide metabolism and metabolic health

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Ceramides are a group of bioactive sphingolipids that exert a wide range of cellular and metabolic effects. Excess accumulation of specific ceramide species is a key feature of obesity progression and is linked to the development of metabolic diseases including insulin resistance and hepatosteatosis. The liver has traditionally been viewed as the predominant synthesiser and exporter of ceramides to metabolic tissues throughout the body. Ceramide metabolism in the small intestine, by contrast, has been poorly characterised. As dietary lipids are, first, absorbed in the gut and distributed via lymphatic channels, we hypothesise that the intestinal-lymphatic route of ceramide synthesis and export is an important mechanism that contributes to whole-body metabolic dysfunction.
Since obesity is caused by an energy imbalance, therapeutic strategies have largely centred upon food intake and/or energy expenditure pathways. Notwithstanding a third, but often overlooked, component to energy balance is nutrient absorption and digestive efficiency. Decreased nutrient absorption is one reported mechanism for the weight loss effect of a diet rich in fibre, while the weight loss drug Orlistat, which inhibits lipase in the gut, works by inhibiting nutrient absorption by approximately 30%. Unfortunately, Orlistat is known to increase defecation and diarrhoea, limiting its therapeutic utility. Nonetheless, targeting pathways associated with lipid and/or carbohydrate absorption, metabolism and packaging to identify drug development targets to combat lipid-associated disorders is the research focus within our laboratory. Accordingly, we have characterised ceramide profiles throughout the intestinal-lymphatic system by adopting a targeted lipidomic workflow and generated a transgenic mouse that conditionally overexpresses the ceramide synthase 2 (CerS2) enzyme in intestinal epithelium. This results in an overexpression of long chain ceramides in the gut. We are currently exploring the hypothesis that manipulation of ceramide production in the intestine may be a new therapeutic target to reduce adiposity and improve glucose metabolism.

An in Vitro Model that recapitulates the Stiff Myocardium and Hypertrophic Cardiomyopathy Disease Progression

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Hypertrophic cardiomyopathy is the most common genetic cardiovascular disorder which is known to have a prevalence of up to 1:500 of the general population. Hypertrophic cardiomyopathy develops from mutations in sarcomere proteins and is characterised by myocyte remodelling, disorganisation of cytoskeletal proteins and altered energy metabolism. It is the leading cause of sudden cardiac death in the young. To understand the role of cardiac stiffness in disease progression, we created an *in vitro* model of hypertrophic cardiomyopathy utilizing hydrogel technology. We isolated cardiac myocytes from the hearts of mice following euthanasia with methoxyflurane followed by sodium pentobarbitone as approved by The University of Western Australia Animal Ethics Committee. We recapitulate the hypertrophic phenotype including the hypermetabolic mitochondrial state by culturing wild-type cardiac myocytes on hydrogels with a Young's Moduli (stiffness) mimicking hypertrophic cardiomyopathy myocardium. Our results mirrored that of myocytes isolated from the Troponin I mutant murine model of human hypertrophic cardiomyopathy (*cTnI-G203S*). Conversely, *cTnI-G203S* myocyte mitochondrial function was completely restored when plated on hydrogels mimicking healthy myocardium. We propose that a mechanosensing feedback mechanism between the extracellular matrix and cytoskeletal network regulates mitochondrial function under healthy conditions, but participates in the progression of hypertrophy resulting from sarcomere gene mutations. We identify alterations in signalling and key 'linker' sites that perpetuate the progression of the hypertrophic phenotype.

From denervation to senescence: exploring the multifaceted signalling in skeletal muscle of geriatric mice

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As global life expectancy continues to climb, maintaining skeletal muscle function is increasingly essential to ensure a good life quality for aging populations. We recently identified mTORC1 activation as a hallmark of sarcopenia, the age-related loss of muscle mass and function. While continuous, long-term treatment with the mTORC1 inhibitor rapamycin predominately exerted anti-sarcopenic effects in mice, we also discovered a pro-aging phenotype and gene expression signature specifically in the gastrocnemius (GAS) muscle. While dosing strategy optimisation may eventually overcome these side effects, the heightened pro-aging gene expression signature also presents a unique opportunity to further characterize the mechanisms contributing to sarcopenia. After initial phenotypic, physiologic and bulk mRNA-seq profiling of these mice (Ham et al., 2020; Ham et al., 2022), we have now generated a large single nuclei (sn) RNA-seq (>100k nuclei) data-set from GAS muscle of 10-month-old adult and 28-month-old control or rapamycin-treated mice fed *ad libitum* or 35% calorie restricted from 19 months of age. Mice were euthanized via sodium pentobarbital (60 mg/kg). Uniform manifold approximation and projections (UMAPs) identified several aging-specific myonuclei clusters, whose prevalence were strongly increased in rapamycin-treated mice. Importantly, the gene expression patterns of these nuclei were distinct from the normal range of myonuclei seen in adult muscle. Further sub-clustering of these ‘Aging’ cluster myonuclei identified a small group of nuclei enriched for genes involved in ‘muscle development’, one enriched for ‘denervation’ markers and another, larger group that displayed both overlapping and distinct marker genes for ‘senescence’ and ‘atrophy’. Importantly, while the gene expression signature of the ‘Aging’ cluster aligned well with the rapamycin-induced pro-aging signature from bulk-seq, each sequencing technique identified candidate genes missed by the other. To link muscle fibre phenotypes with the presence of ‘Aging’ cluster signatures, we now use highly multiplexed single molecule (sm) RNA-fluorescence *in situ* hybridization (FISH) to visualize the expression of genes associated with ‘denervation’, ‘senescence’ and ‘atrophy’ clusters in GAS muscle cross sections. In summary, our results support the notion that local expression changes in multiple genes contribute to the initiation of sarcopenia and they highlight the likely need for multiple future treatment strategies.

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Understanding the role of Starch Binding Domain containing protein 1 (STBD1) in maintaining cardiac function

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Diabetic cardiomyopathy is characterised by metabolic stress and a disturbance in insulin-glucose signalling. Recently, cardiomyocyte glycogen handling disturbances have been identified in diabetes. Increased myocardial glycogen accumulation has been observed clinically in diabetic patients and in rodent models of diabetes (Delbridge *et al.,* 2017). Evidence from study of glycogen storage diseases indicates that autophagic processes are involved in regulating cardiomyocyte glycogen levels.
A glycogen-selective autophagy pathway, glycophagy, has recently been identified and some evidence indicates that cardiomyocyte glycolysis is dependent on lysosomal glycogen degradation (Delbridge *et al.,* 2022). Understanding of this non-canonical pathway of glycogen handling is currently limited. Our work has identified that Starch Binding Domain containing protein 1 (STBD1) is a 'glycophagy receptor' in cardiomyocytes which tags glycogen for localization to autophagosomes for degradation (Koutsifeli et al., 2022). The role of STBD1 in myocardial physiology and pathology is yet to be determined. The aim of this study was to investigate the role of STBD1 in maintaining cardiac function and examine whether genetic deletion of STBD1 impacts functional and metabolic responses in female and/or male rodents.
An STBD1 knockout (KO) model was generated using CRISPR/Cas9, in C57Bl6J mice (wild type, WT). Echocardiography was performed under light anaesthesia (inhalation of isoflurane at 1.5%). A glucose tolerance test involving a six hour fast prior to a bolus injection of glucose (1.5 g/kg, i.p.) was then performed. Subsequently, mice were euthanised with pentobarbital (20 mg/kg, i.p.) and cardiac tissue was collected to measure glycogen content via an enzymatic assay.
At 30 weeks of age, relative to sex matched WT, female STBD1 KO mice exhibited a greater reduction in ejection fraction than STBD1 KO males (-33% and -16%, respectively, $p < 0.05$). Relative to male counterparts, all females had reduced stroke volume regardless of genotype and only female STBD1 KO mice had reduced fractional shortening (-27% vs female WT, $p < 0.05$). Mitral valve deceleration time was reduced in both STBD1 KO male (-41% vs male WT, $p < 0.05$) and female (-59% vs female WT, $p < 0.05$) mice. Furthermore, E/e' was higher in both male and female STBD1 KO mice when compared to their wild-type counterparts (+67% and +65%, respectively, $p < 0.05$). Male mice showed indication of glucose intolerance when compared to females, and there was no STBD1 genotype difference. Female STBD1 KO mice had lower myocardial glycogen content compared to their respective wild-type controls (-42%, $p < 0.05$), while male glycogen levels were not genotypically different.
This study provides evidence that STBD1 deficiency is associated with cardiac dysfunction which is exacerbated in females and linked with lower myocardial glycogen content. Further understanding of the involvement of STBD1 in regulating sex-specific aspects of cardiac function and glycaemic control is required.

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The molecular athlete: From molecules to medals

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Skeletal muscle demonstrates a remarkable plasticity capable of adapting to a variety of external stimuli including the habitual level of contractile activity (physical activity versus inactivity), loading state (endurance- versus resistance-based exercise), substrate availability (predominantly carbohydrate- and fat-based fuels), and the prevailing environmental conditions (thermal stress). While this phenomenon of plasticity is common to all vertebrates, there exists a wide variation in the magnitude of adaptability among species and between individuals within a species (i.e., athletes versus untrained persons).
Exercise training represents a repeated challenge to whole-body homeostasis provoking widespread perturbations in numerous cells, tissues, and organs that are caused by or are a response to the increased metabolic activity of contracting skeletal muscles. To meet this challenge, multiple integrated and often redundant responses operate to blunt the homeostatic threats generated by exercise-induced increases in muscle energy and oxygen demand. These involve a series of highly coordinated reactions that fundamentally involve increased expression and/or activity of key proteins, mediated by an array of signalling events, pre- and post-transcriptional processes, regulation of translation and protein expression, and modulation of protein (enzyme) activities and/or intracellular localization. There are multiple stimuli associated with endurance- and resistance-based exercise training, various signalling kinases that respond to these divergent stimuli, and numerous downstream pathways and targets of these kinases. In this talk I will discuss how the application of molecular biology techniques to exercise science has enabled sports scientists to unravel some of events underpinning ‘the molecular athlete.’

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Survival of the sleepiest: How sleep deprivation impacts athlete physiology and performance

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Background
Sleep is essential for physiological restoration in athletes. However, athlete sleep is regularly compromised by morning training, night competition, or travel. The negative impact of short-term sleep deprivation or sleep restriction on acute performance (e.g., muscle strength, time to exhaustion, reaction time, etc) and physiological (e.g., anabolic and catabolic hormone responses, muscle protein metabolism) measures is relatively well understood in males[1,2]. Our group investigated the functional and physiological response of the muscle to resistance exercise (REx) with sleep restriction, 1) over time and, 2) in females.
Methods
Ten healthy, resistance-trained, eumenorrheic females aged 18-35 years undertook a randomised cross-over study of nine nights' sleep restriction (SR; 5-h time in bed) and normal sleep (NS; ≥7 h time in bed) with a minimum 6-week washout. Participants strength was assessed over four REx sessions per trial (day 3, 5, 7 and 9). Muscle biopsies were collected pre- and post-REx on days 3 and 9. Blood samples were collected pre- and post-REx on days 3, 5, 7 and 9, with four additional blood samples collected every two waking hours on days 3 and 9 for anabolic and catabolic hormone analysis. Waking saliva samples were collected daily. Gene and protein expression were assessed by RT-PCR and Western Blot, respectively.
Results
Volume-load decreased trivially (<1%, $p < 0.05$) with SR. Mean concentric velocity per set was slower during SR for lower body (up to 15%, $p < 0.05$), but not upper body, compound lifts. SR increased salivary cortisol area under the curve (by 42%, $p < 0.05$). No change in testosterone, cortisol or SHGB was observed with sleep condition or days. Gene expression of markers of circadian regulation (CRY1/2, PER2, BMAL1) and protein degradation (Atrogin, MURF1, FOXO1/3) were downregulated following three days of SR (all $p < 0.05$). After nine days, circadian markers returned to NS levels, however all protein degradation markers increased above NS levels (all $p < 0.05$).
Conclusion
Adequate sleep is critical to maintaining physical performance and metabolic markers at an optimal level. Our findings suggest that markers of exercise quality and internal load may be more sensitive than volume-load to advise coaches to the decline in resistance exercise performance for females experiencing sleep restriction. Further research is needed to determine if the negative trends in circadian and metabolic markers identified in our study are maintained long-term and to understand the consequent implications for metabolic and immune health. In practice, coaches should educate athletes, modify training schedules or programs and implement sleep or performance enhancement strategies ahead of known periods of sleep loss.

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TRPM4-Piezo1 signalling axis drives pressure overload-induced cardiac hypertrophy

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Pathological left ventricular hypertrophy (LVH) occurs in response to pressure overload and remains the single most important clinical predictor of cardiac mortality. The molecular pathways in the induction of pressure overload LVH are potential targets for therapeutic intervention. Current treatments aim to remove the pressure overload stimulus for LVH, but do not completely reverse adverse cardiac remodelling. Although numerous molecular signalling steps in the induction of LVH have been identified, the initial step by which mechanical stretch associated with cardiac pressure overload is converted into a chemical signal that initiates hypertrophic signalling remains unresolved.

In this study, we investigate the role of the transient receptor potential melastatin 4 (TRPM4) channel in pressure overload LVH induced by transverse aortic constriction (TAC). We compare the results between homozygous *Trpm4* cardiomyocyte-specific knock-out mice and wild type (WT) control mice and demonstrate that loss of cardiomyocyte TRPM4 significantly attenuates the development of LVH observed in response to TAC in WT mice. This effect is associated with reduced activation of the Ca^{2+}-dependent hypertrophic signalling pathway responding to pressure overload. In addition, we employed the HL-1 mouse atrial myocyte-like cell line as an *in vitro* model to investigate the interaction of TRPM4 and a potential upstream mechanosensitive channel Piezo1 which can be activated by mechanical stretch. We show that when Piezo1 is activated, TRPM4 is stimulated by the resulting increase of intracellular Ca^{2+} which causes an increase in action potential frequency in HL-1 cells.

Our results suggest that TRPM4 channel is an important component of the mechanosensory signalling pathway that induces LVH in response to pressure overload and represents a potential novel therapeutic target for the prevention of pathological LVH. We also provide *in vitro* evidence of a functional interaction between Piezo1 and TRPM4 in a cardiomyocyte-like cell line. This model in which a Ca^{2+}-activated ion channel acts as a downstream effector or amplifier of a primary mechanosensitive current is likely to be ubiquitous within mechanosensory pathways.

Polyamine metabolism regulates muscle stem cell and fibro-adipogenic progenitor proliferation and differentiation

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Skeletal muscle regeneration is a highly complex and coordinated process involving a variety of cell populations. Muscle stem cells (MuSCs) undergo several stages of proliferation and differentiation to facilitate tissue repair and fibroadipogenic progenitors (FAPs) support the MuSC response (Fiore *et al*., 2016). Previous studies have identified metabolism as a key determinant in cell state and lineage progression (Lunt & Vander Heiden, 2011; 2015; Pala et al., 2018). We conducted untargeted steady-state metabolomics and identified key intermediates in the polyamine synthesis pathway (particularly putrescine), as some of the most differentially abundant metabolites after barium chloride and ischaemia reperfusion injury. The importance of this metabolic pathway in the process of skeletal muscle regeneration is unknown. We therefore sought to characterise and determine the role of polyamine metabolism in two key cell populations, MuSC and FAPs.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). MuSCs were isolated from hindlimb muscles of 12-week-old $Pax7^{creERT2}$; $R26R^{YFP}$ mice and $CD45^{-}/CD31^{-}/\alpha7^{-}$ integrin/$Sca1^{+}$ FAPs from C57BL/6 mice by fluorescent activated cell sorting (FACS). All mice were killed by rapid cervical dislocation. Primary cells and C2C12 immortalised murine myoblasts were cultured to assess the effect of either activating polyamine metabolism via supplementation with pathway metabolites ornithine or putrescine, or inactivating polyamine metabolism by inhibiting key enzymes in the pathway with α-difluoromethylornithine (DFMO) or deoxyhypusine synthase inhibitor (GC7), on cell proliferation and differentiation. A combination of raw cell counts, enzyme activity assays, immunofluorescence and immunoblotting were performed.

C2C12s and MuSCs proliferated at a greater rate after supplementation with either 100 μM ornithine ($P<0.0001$) or 500 μM putrescine ($P<0.001$) with a greater total cell number following 72 hours and reduced mean doubling time. Ornithine, but not putrescine, promoted FAP proliferation ($P<0.001$). DFMO alone reduced proliferation of all three cell types in a dose-dependent manner ($P<0.0001$). Importantly, the increase in C2C12 proliferation with ornithine supplementation was prevented when combined with 100 μM DFMO ($P<0.001$). GC7 impaired proliferation of all three cell types in a dose-dependent manner ($P<0.0001$).

Ornithine had no effect on either C2C12 or MuSC differentiation, while 500 μM putrescine promoted myotube formation after 4 days of differentiation ($P<0.0001$). DFMO alone impaired differentiation, but this was rescued when combined with 500 μM putrescine ($P<0.05$). Supplementation with ornithine or putrescine had no effect on FAP cell differentiation into adipocytes over 7 days. Treatment with DFMO impaired FAP adipogenic differentiation ($P<0.001$). GC7 severely inhibited myotube formation in C2C12s and MuSCs ($P<0.0001$). Moreover, GC7 inhibited adipogenic differentiation in FAPs and instead induced a fibroblast phenotype ($P < 0.0001$).

These results demonstrate the importance of the polyamine synthesis pathway in both immortalised C2C12 myoblasts and primary MuSCs and FAPs *in vitro*. Follow-up studies will investigate the role of these metabolites and inhibitors *in vivo* after muscle injury.

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TMEM161B is required for the maintenance of cardiac rhythm

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Introduction: Long QT syndrome (LQTS) is caused by mutations in cardiac ion channels, which can cause arrhythmias and sudden cardiac death. We identified TMEM161B as a novel regulator of cardiac rhythm whereby *tmem161b* mutant zebrafish present with LQTS-like phenotypes: skipped/irregular ventricular beats, bradycardia, and prolonged repolarization linked to increased I_{Kr} and $I_{Ca,L}$ currents. However, the requirement of TMEM161B in cardiac rhythm maintenance is unknown as loss of *Tmem161b* in mice results in early lethality. Therefore, this study examined whether TMEM161B plays a maintenance role in cardiac rhythm by deleting it systemically in adult mice.

Methods: To ablate *Tmem161b* systemically, we used a tamoxifen-inducible ubiquitous Cre-recombinase line crossed with a newly generated Tmem161b$^{flox/flox}$ line. Cre activity was induced in 12-week of old male Tmem161b$^{flox/flox}$ (WT), Tmem161b$^{flox/+}$:Ubc$^{Cre/ERT2}$ (Het), and Tmem161b$^{flox/flox}$:Ubc$^{Cre/ERT2}$ (KO) mice. Cardiac function was assessed with conscious ECGs and echocardiography, body composition measured with EchoMRI, and behaviour was video recorded. At ~6 weeks post tamoxifen, mice were killed for cardiac collection. In parallel, we performed pulldown experiments to investigate what TMEM161B physically interacts with. Transgenic zebrafish expressing Tmem161b-GFP fusion proteins were used: adult zebrafish hearts were dissected, and pulldowns performed. Tmem161b-GFP binding partners were identified using LC-MS/MS.

Results: KO males rapidly lost body weight after tamoxifen administration; -5% at 3 weeks, -10% at 4 weeks, and -13% at 5 weeks compared to their starting weight. One-week post-tamoxifen induction cardiac electrical activity was unchanged. Whereas there were overt conduction changes in KO mice 5-weeks post-tamoxifen, suggesting a progressive degeneration in cardiac function: reduced heart rate (-6%) and increased QTcH duration (+6%), QT dispersion (+27%), ST duration (+7%), and heart rate variability (+100%). At postmortem, no changes in relative heart or ventricle weights were observed. Histological analysis identified no changes in cardiomyocyte size, collagen deposition, or glycogen content, with lipid content analysis currently underway.

LC-MS/MS identified 54 TMEM161B binding partners including the sarcoplasmic reticulum Ca^{2+} channels, RYR2 and SERCA2; suggesting that TMEM161B may directly modulate ion channels. To further validate this, we are examining total and phosphorylated cardiac SERCA2, RYR2, and PLB protein content in our mice via Western blotting. We are also exploring genetic interactions of TMEM161B with RYR2 and SERCA2 by crossing our Tmem161b$^{Del/+}$ mice with either Ryr2$^{Del/+}$ or Serca2$^{Del/+}$ mice and performing conscious ECGs at various ages (3, 6, 9, and 12-weeks of age); experiments and analysis of which are currently underway.

Conclusion: This study demonstrates that TMEM161B loss-of-function in the adult results in a progressive loss of cardiac rhythm over time, degenerating to a phenotype consistent with LQTS, wh8ch is similar to what we observed in zebrafish and mouse embryos. This work highlights that TMEM161B is required for cardiac rhythm maintenance and mutations in this gene may contribute to LQTS.

Using 'omics' to understand mitochondrial adaptations to different types of exercise

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Given the importance of mitochondrial biogenesis for skeletal muscle performance and health, considerable attention has been given to understanding the molecular changes that help to determine mitochondrial adaptations to exercise. This talk will focus on published and unpublished 'omics data that provide new and exciting insights into the many molecular changes that contribute to exercise-induced mitochondrial adaptations. The results of RNA sequencing (RNA-seq) based transcriptomics highlight how different mitochondria-related gene transcripts respond following different types of exercise, and how this may help to explain divergent mitochondrial adaptations to different types of exercise training. These RNAseq results also indicate that there are transcriptional responses that are shared across different exercise prescriptions. Of particular interest is how exercise-induced mitochondrial damage activates transcriptional pathways associated with mitochondrial stress and how this may help to explain the powerful effects of very high-intensity exercise to improve mitochondrial respiratory function. The results of training studies incorporating whole-muscle proteomics will then be used to highlight an intricate and previously unknown network of differentially prioritised mitochondrial adaptations that occur in response to different types of training. It will be shown that changes in hundreds of transcripts, proteins, and lipids are not stoichiometrically linked to the overall increase in mitochondrial content. Finally, the results of single-fibre proteomics show how exercise intensity influences fibre recruitment and ultimately induces fibre-specific changes in mitochondrial proteins that can help to explain how different types of exercise induce divergent mitochondrial adaptations. Finally, this presentation will highlight how these exciting new tools can help exercise and sport scientists to better understand how best to prescribe exercise to achieve specific mitochondrial adaptations. In summary, this session will provide an important update on how different physiological stresses help to stimulate mitochondrial adaptations.

Therapeutic effect of β-Hydroxy-β-Methylbutyrate supplementation on the *mdx* mouse phenotype

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INTRODUCTION: Duchenne muscular dystrophy (DMD) is one of the most severe forms of inheritable muscular dystrophies, caused by a genetic mutation resulting in the loss of full-length dystrophin. This loss of full-length dystrophin initiates a cascade of negative mechanistic changes, consisting of both chronic inflammation and oxidative stress, culminating in skeletal muscle atrophy and a decreased force production. Recent literature has highlighted the potential for the leucine metabolite β-Hydroxy-β-methylbutyrate (HMB) as a treatment option for those with DMD, primarily through its ability to attenuate skeletal muscle atrophy. Specifically, HMB increases protein synthesis through the upregulation of mTORC1 signalling, whilst subsequently decreasing protein degradation via inhibition of the ubiquitin proteasome system (1). Moreover, HMB has also shown to inhibit the inflammatory pathway nuclear factor kappa B (NF-kB), a pathway that is upregulated in DMD (2). The pre-clinical *mdx* DMD mouse model also contains a genetic mutation resulting in a loss of full-length dystrophin, coinciding in similar skeletal muscle changes, such as chronic inflammation, increased NF-kB, and a reduced skeletal muscle force output. However, to date, no study has investigated HMB's potential therapeutic effect on *mdx* skeletal muscle function.
METHODS: Three-week-old C57BL/10mdx (*mdx*) (n=6-12) and C57BL/10ScSn wild-type (WT) (n=8-12) control mice were given drinking water with or without 500mg/kg of HMB daily for 3 weeks. At 6-weeks of age, mice underwent a hanging wire test, coupled with both a forelimb and full-body grip strength test to assess both muscle endurance and force production. Once completed, mice were anaesthetised (2-4% isoflurane) and both the hindlimb extensor digitorum longus (EDL) and soleus (SOL) muscles were dissected to assess *ex vivo* contractile properties. Animal experimentation was approved by the Victoria University (VU) Animal Ethics Committee (AEC 22/005) and performed in accordance with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.
RESULTS: HMB supplementation induced an increase in *mdx* hanging wire time, which coincided with an increase in *mdx* holding impulse, with no effect observed on WT mice. WT mice demonstrated both a higher forelimb and full body grip strength compared to the *mdx* groups, with HMB inducing an increase to both forelimb and full body grip strength within the *mdx* mice. HMB also improved *mdx* forelimb fatigue index with no effect on the WT groups. The *mdx* EDL muscles also exhibited a HMB induced increase in muscle mass when normalised to body mass despite there being no significant difference within the SOL muscles. Increased EDL muscle mass coincided with an increase in absolute force of HMB treated *mdx* EDL muscles, although there was no change in HMB treated *mdx* EDL specific force. No significant difference was observed within the HMB treated SOL absolute and specific forces, indicating a potential preference for HMB treatment on the fast-twitch EDL muscles. WT EDL and SOL muscles exhibited both a higher absolute and specific force when compared to *mdx* groups.
CONCLUSION: Combined, these data suggest that HMB supplementation positively influences the *mdx* phenotype, with the fast-twitch EDL muscle being more sensitive to treatment compared to the slow-twitch SOL, supporting further work to determine the efficacy of HMB as a potential therapeutic adjunct for DMD treatment.

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Unveiling the Role of Reactive Astrogliosis in Neurofibrosis-Driven Metabolic Disease: Implications for Obesity and Type-2 Diabetes

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Background:
Neurofibrosis, characterised by pathological remodelling of extracellular matrix around neurons in the arcuate nucleus of the hypothalamus (ARC), plays a pivotal role in the progression of metabolic diseases. It exacerbates obesity and type-2 diabetes by inducing cellular insulin resistance within ARC neurons, leading to profound whole-body metabolic dysfunction. Although the causal link between ARC neurofibrosis and metabolic disease has recently been established, the precise cellular and molecular mechanisms underlying neurofibrosis remain unexplored.

Methods*/Results:
We investigated the involvement of astrocytes in neurofibrosis, considering that these cells express several proteoglycan and glycosaminoglycan components of neurofibrosis. We hypothesised that neurofibrosis may originate from reactive changes in ARC astrocytes during metabolic disease progression. Our study revealed that reactive astrogliosis within the ARC positively correlates with the development of neurofibrosis from 4 weeks of high-fat, high-sugar feeding with a large proportion of reactive astrocytes situated within the pathogenic extracellular matrix in obese mice. Using Airyscan super-resolution microscopy, we revealed that obesity leads to a profound increase in interactions between reactive astrocytes and extracellular matrix within the ARC. This suggests a relationship between reactive astrogliosis and neurofibrosis in the ARC that could underlie metabolic disease progression.

To establish a direct link between reactive astrogliosis and neurofibrosis, we developed a chemogenic tool based on Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) technology to induce reactive astrogliosis in the ARC of lean mice. This induction resulted in a marked development of neurofibrosis within the ARC, leading to impaired glucose homeostasis in otherwise healthy mice. Interestingly, these impairments in glycaemic control were rescued by enzymatic digestion of the neurofibrosis extracellular matrix, confirming that the effects depended on the astrocyte-induced extracellular matrix remodelling and not intrinsic astrocytes *per se*.

To investigate the potential for reversing obesity-driven neurofibrosis by inhibiting reactive gliosis, we chemogenetically attenuated reactive astrogliosis within the ARC of diet-induced obese mice using DREADD-hM4Gi expressing in reactive astrocytes of the ARC. Our findings demonstrate that modulating reactive astrogliosis in the ARC can reverse neurofibrosis, leading to remission from diet-induced obesity.

Conclusion:
Our results uncover a previously unrecognised mechanism by which reactive astrogliosis in the ARC drives the development of metabolic disease through the pathogenesis of neurofibrosis. This discovery holds significant implications for understanding and potentially treating obesity and type-2 diabetes by targeting neurofibrosis and its associated astrocyte-mediated mechanisms in the hypothalamus.

*All mice were placed under anesthesia by isoflurane inhalation via nose cones on stereotaxic frames prior to surgery.

The role of cardiomyocyte cavin-1 in cardiac function and development

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Membranous signalling domains (termed caveolae) and associated caveolin and cavin proteins govern myocardial responses to mechanical and ischemic stress, while abnormalities in these proteins are implicated in different cardiac disorders. We previously identified that global cavin-1 deficiency causes diastolic dysfunction; an effect reversible with nitric oxide synthase inhibition and mimicked by a nitric oxide donor (Kaakinen, Reichelt et al. 2017). Cavin-1 is expressed in different cardiac cell types including cardiomyocytes, fibroblasts, leukocytes and endothelial cells (Quaife-Ryan, Sim et al. 2017), and the relative contributions of these cell types to the regulation of cardiac function is currently unknown. Therefore, this study aimed to delineate the specific role of cardiomyocyte cavin-1 in regulating cardiac function and development. We generated a cavin-1 flox mouse line using CRISPR, and used an adeno-associated virus serotype 9 (AAV9) with a chicken troponin T promoter to drive expression of cre recombinase in cardiomyocytes (AAV9;cTNT-iCre). P1 Cavin-1 wild-type (cavin-1^{ww}) and cavin-1 flox (cavin-1^{ff}) neonatal mice were anaesthetised by placing them on ice for 60 seconds and AAV9;cTNT-iCre was administered via a temporal vein injection ($6.67x10^{10}$vgc/neonate, 30-gauge needle). Neonatal mice were then returned to their mothers after recovering under a warming lamp. A subset of neonatal mice were culled (by decapitation) at P7 with RT-qPCR used to confirm successful deletion of cavin-1. Molecular analyses of whole heart tissue via RT-qPCR confirmed that cavin-1 gene expression was downregulated by 40% in AAV-injected cavin-1^{ff} mice, with no significant changes in expression of *Cav1* (caveolin-1) and *Cav3* (caveolin-3), *NOS 2* (iNOS) and *NOS 3* (eNOS) gene expression. Interestingly, the downregulation of cavin-1 was coincident with reduced atrial natriuretic peptide (ANP). Cavin-1 deletion in cardiomyocytes resulted in diastolic dysfunction (E/E' as assessed by echocardiography) at P14, with mice having smaller body weight than wild-type neonates despite maintaining comparable heart sizes. Despite this, mice survived to adulthood. Ongoing experiments aim to: measure protein expression; identify the effects of long-term cardiomyocyte cavin-1 deletion on cardiac maturation; and measure vascular and contractile function in Langendorff-perfused adult hearts. In conclusion, cardiomyocyte cavin-1 impacts cardiac function in the postnatal period by the cavin/caveolae/NO axis.

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Targeting hepatic INPP1 for the treatment of non-alcoholic fatty liver disease

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Despite the rising prevalence of non-alcoholic fatty liver disease (NAFLD), there is an absence of FDA-approved treatments for liver fibrosis and non-alcoholic steatohepatitis (NASH). This gap in therapeutic options places patients with NAFLD at an elevated risk of developing liver cancer, liver failure, and overall diminished life expectancy. The progression of NAFLD, including the major hallmarks of defective lipid metabolism, inflammation, and fibrosis, are not uniform across individuals. Beyond environmental factors, genetic variations, specifically single nucleotide polymorphisms (SNPs), play a significant role in dictating the onset and intensity of NAFLD and liver fibrosis. A recent study from our team evaluated the susceptibility to western diet-induced NASH in eight common inbred mouse strains, aimed at identifying mouse strains susceptible or resistant to dietinduced NASH and liver fibrosis. Given the genetic variability between these mouse strains, this analysis further provided the chance to perform quantitative trait loci (QTL) mapping to identify novel genetic variants that might influence the observed mouse strain-specific phenotypes, particularly in the context of susceptibility/resistance to NASH. This mouse strain comparison identified the BALB/c mouse strain as completely protected from hepatic steatosis and any features of NASH or hepatic fibrosis, suggesting a potential genetic profile of ‘resistance to NASH’. To corroborate this idea, QTL mapping identified significant BALB/c specific cis-regulation of the proteome. Specifically, we identified a BALB/c-specific SNP in INPP1 (inositol polyphosphate 1 phosphatase), that may confer protection against diet-induced NASH. INPP1 is a Mg(2+)-dependent phosphatase that catalyzes the hydrolysis of the 1-position phosphate from inositol 1,4-bisphosphate and inositol 1,3,4-trisphosphate and participates in inositol phosphate metabolism. It might have the potential to impact on the intracellular diglyceride and calcium release. Little is known about the role of INPP1 in ectopic lipid accumulation in the liver and NAFLD progression. Subsequent in vitro experiments in HepG2 liver cells showed that CRISPR-mediated knockdown of INPP1 decreased triglyceride accumulation which was associated with a substantial decrease in fatty acid uptake and de novo lipogenesis. Decreased lipid accumulation was present despite a significant reduction in fatty acid and glucose oxidation following INPP1 knockdown. Together, this study indicates that INPP1 may be a novel regulator of hepatic fatty acid uptake and lipogenesis, and that increasing hepatic INPP1 expression may associate with the development of NAFLD.

Mitochondrial redox regulation in adaptation to exercise

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Reactive oxygen species (ROS) regulate exercise responses and adaptations in skeletal muscle. While the major sources of exercise-induced ROS appear to be non-mitochondrial, there is evidence of acute exercise-induced changes in the mitochondrial redox environment. The role of mitochondrial ROS in signalling exercise responses and adaptations has not been investigated in detail. The mitochondria-targeted antioxidant MitoQ has been shown to decrease skeletal muscle mitochondrial ROS levels and therefore provides a potential tool to investigate the role of mitochondria in exercise-induced redox signalling. We investigated the effect of MitoQ supplementation on a) the skeletal muscle mitochondrial and antioxidant gene transcriptional response to acute high-intensity exercise and b) skeletal muscle mitochondrial content and function following exercise training. In a randomised, double-blind, placebo-controlled, parallel design study, 23 untrained men (age: 44 ± 7 years, VO_{2peak}: 39.6 ± 7.9 ml/kg/min) were randomised to receive either MitoQ (20 mg/d) or a placebo for 10 days before completing a bout of high-intensity interval exercise (cycle ergometer, 10 × 60 s at VO_{2peak} workload with 75 s rest). Blood samples and vastus lateralis muscle biopsies were collected before exercise and immediately and 3 h after exercise. Participants then completed high-intensity interval training (HIIT; 3 sessions per week for 3 weeks) and another blood sample and muscle biopsy were collected. There was no effect of acute exercise or MitoQ on systemic (plasma protein carbonyls and reduced glutathione) or skeletal muscle (mtDNA damage and 4-HNE) oxidative stress biomarkers. Acute exercise-induced increases in skeletal muscle peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) mRNA expression were augmented in the MitoQ group. Despite this, training-induced increases in skeletal muscle mitochondrial content were similar between groups. HIIT-induced increases in VO_{2peak} and 20 km time trial performance were also similar between groups while training-induced increases in peak power achieved during the VO_{2peak} test were augmented in the MitoQ group. These data suggest that training-induced increases in peak power are enhanced following MitoQ supplementation, which may be related to the augmentation of skeletal muscle PGC1α expression following acute exercise. However, these effects do not appear to be related to an effect of MitoQ supplementation on exercise-induced oxidative stress or training-induced mitochondrial biogenesis in skeletal muscle.

Dietary vitamin B_{12} deficiency reduces white adipose tissue expression of insulin signalling genes in female rats

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INTRODUCTION. Many studies report a higher prevalence of diabetes mellitus among B_{12} deficient individuals, but the nature of this relationship remains incompletely understood. Thus, it is unclear if B_{12} deficiency can directly contribute to the development of diabetes mellitus, or if diabetes mellitus or anti-diabetic medications cause B_{12} deficiency.

In female rats, we previously showed that 1 month of dietary B_{12} deficiency led to a prediabetic-like phenotype characterised by glucose intolerance and a delayed rise in plasma insulin levels early after glucose injection (Neal et al., 2023). Random blood glucose levels, fasting glucose, insulin and glucagon levels, and HOMA-IR were all unaffected. However, HOMA-β was lower, which suggested potentially reduced beta cell activity and/or function at baseline. That study focused largely on characterising the metabolic phenotype in our model. Tissue analyses were focused only on liver, where in terms of insulin signalling, only mRNA levels were assessed and only a slight effect on *Irs1* mRNA levels (+23%) was found. In the present work, we aimed to determine if deficits in insulin signalling and/or glucose transport in skeletal muscle and adipose tissue contributed to the metabolic phenotype we observed.

METHODS. We used tissues collected from the same cohort of rats previously studied in Neal et al. (2023). Briefly, 10 week old female Sprague-Dawley rats were acclimatised for >1 week and then fed control (n = 13, ~95 µg/kg B_{12}) or B_{12} deficient (n = 14, ~5 µg/kg B_{12}) diets for 1 month. This achieved ~3- and 17-fold reductions in plasma and liver B_{12} levels, respectively, compared to control diet-fed rats (Neal et al., 2023). On day 28, food was removed for 5-7 hours before rats were anaesthetised with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) (1 ml/kg, i.p.) and killed via cardiac puncture. Gastrocnemius muscle and retroperitoneal white adipose tissue (rWAT) was collected, frozen in liquid nitrogen and stored at -80°C until analysis. mRNA expression was determined using qPCR (gastrocnemius muscle, n = 13-14; rWAT, n = 12-14).

RESULTS. In gastrocnemius muscle, B_{12} deficiency did not alter the mRNA expression of any insulin signalling gene examined (*Insr*, *Irs1*, *Pik3ca*, *Pik3r1*, *Akt1* or *Gsk3b*). Similarly, no changes were seen in *Slc2a4 (Glut4)*, *Tbc1d4* (encoding AS160, involved in insulin-stimulated translocation of GLUT4 to the plasma membrane) or *Slc2a1 (Glut1)*. In contrast, in rWAT, reduced mRNA levels of *Insr*, *Irs1*, *Pik3r1*, *Akt1* and *Gsk3b* were found (-35 to -64%, all $p < 0.05$). Although *Slc2a4* mRNA levels appeared unchanged in rWAT, *Tbc1d4* mRNA levels were reduced by 33% ($p < 0.05$), which suggests that insulin-dependent glucose uptake in this tissue could be impaired. Interestingly, these changes were linked to decreased mRNA levels of the B_{12}-dependent enzymes *Mtr* and *Mut* (-39% and -22%, both $p < 0.05$) in rWAT, while in gastrocnemius muscle, both were unchanged at the mRNA level.

CONCLUSION. Our findings suggest that deficits in insulin signalling and potentially insulin-dependent glucose uptake in rWAT may contribute to the glucose intolerant phenotype we have previously observed in this B_{12} deficient rat model. These results support the idea that B_{12} deficiency may directly contribute to the onset of metabolic disorders like diabetes mellitus. Protein levels and concentrations of B_{12} and related metabolites in gastrocnemius muscle and rWAT are currently being investigated.

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HDAC11 Inhibition Blocks Adipocyte Lipolysis Through Reversible Myristoylation of HSL

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In obesity, the storage capacity of visceral and subcutaneous adipose tissue (AT) depots is limited, leading to dyslipidemia - excess circulating lipids – and fat accumulation in ectopic tissues if excess caloric intake persists. Excess fat deposition in peripheral organs, such as the liver and heart, causes lipotoxicity and metabolic disorders including non-alcoholic fatty liver disease, type II diabetes and diastolic dysfunction respectively, accompanied by systemic low-grade inflammation. There is an ongoing obesity pandemic, with nearly half of adults in the U.S. with a B.M.I. > 30 kg/m2, and global obesity statistics not far behind. Accordingly, associated major socioeconomic health burdens, including type II diabetes and heart disease, are rising. Along with obesity, metabolic syndrome is described as a hallmark of aging. The World Health Organization projects that the world's population of people aged 80 years or older is expected to triple between 2020 and 2050 to reach 426 million. Together, the obesity pandemic and aging population, make metabolic disease a major socioeconomic health burden of the coming decades.

Published work from our laboratory has demonstrated that global loss of the poorly characterized class IV histone deacetylase, HDAC11, in mice protects against chronic high fat high sugar (HFHS) diet-driven metabolic dysfunction, including improved glucose tolerance, elevated leptin and reduced circulating lipids and less fat deposition in the liver. HDAC11 KO mice also have lower expression of pathological gene markers in left ventricular heart tissue compared with WT mice following a year of HFHS feeding. HDAC11 is known to have very weak histone deacetylase activity and is in fact a potent lysine demyristoylase (14-carbon fatty acid modification).

Using a combination of pharmacological, genetic and molecular cloning approaches, we have extensive evidence to support hormone sensitive lipase (HSL) as a novel HDAC11 demyristoylase substrate and have identified a single highly conserved lysine in the hinge region of HSL that is the site of myristoylation. HDAC11 inhibition in rodent and human adipocytes blunts catecholamine-stimulated lipolysis, which is dependent on myristoylation of lysine 239 in adipocyte HSL. Furthermore, HDAC11 inhibition increases phosphorylation at the inhibitory serine 565 AMPK phosphorylation site on HSL. We continue to explore the role of HSL myristoylation in attenuation of HSL activity and lipolysis and the effects on peripheral organ health under conditions of chronic catecholamine stimulation, such as obesity.

Title: HDAC11 Inhibition Blocks Adipocyte Lipolysis Through Reversible Myristoylation of HSL

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In obesity, the storage capacity of visceral and subcutaneous adipose tissue (AT) depots is limited, leading to dyslipidemia - excess circulating lipids – and fat accumulation in ectopic tissues if excess caloric intake persists. Excess fat deposition in peripheral organs, such as the liver and heart, causes lipotoxicity and metabolic disorders including non-alcoholic fatty liver disease, type II diabetes and diastolic dysfunction respectively, accompanied by systemic low-grade inflammation. There is an ongoing obesity pandemic, with nearly half of adults in the U.S. with a B.M.I. > 30 kg/m2, and global obesity statistics not far behind. Accordingly, associated major socioeconomic health burdens, including type II diabetes and heart disease, are rising. Along with obesity, metabolic syndrome is described as a hallmark of aging. The World Health Organization projects that the world's population of people aged 80 years or older is expected to triple between 2020 and 2050 to reach 426 million. Together, the obesity pandemic and aging population, make metabolic disease a major socioeconomic health burden of the coming decades.

Published work from our laboratory has demonstrated that global loss of the poorly characterized class IV histone deacetylase, HDAC11, in mice protects against chronic high fat high sugar (HFHS) diet-driven metabolic dysfunction, including improved glucose tolerance, elevated leptin and reduced circulating lipids and less fat deposition in the liver. HDAC11 KO mice also have lower expression of pathological gene markers in left ventricular heart tissue compared with WT mice following a year of HFHS feeding. HDAC11 is known to have very weak histone deacetylase activity and is in fact a potent lysine demyristoylase (14-carbon fatty acid modification).

Using a combination of pharmacological, genetic and molecular cloning approaches, we have extensive evidence to support hormone sensitive lipase (HSL) as a novel HDAC11 demyristoylase substrate and have identified a single highly conserved lysine in the hinge region of HSL that is the site of myristoylation. HDAC11 inhibition in rodent and human adipocytes blunts catecholamine-stimulated lipolysis, which is dependent on myristoylation of lysine 239 in adipocyte HSL. Furthermore, HDAC11 inhibition increases phosphorylation at the inhibitory serine 565 AMPK phosphorylation site on HSL. We continue to explore the role of HSL myristoylation in attenuation of HSL activity and lipolysis and the effects on peripheral organ health under conditions of chronic catecholamine stimulation, such as obesity.

Interrogating the therapeutic potential of Hexosaminidase A in advanced liver disease

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The prevalence of non-alcoholic steatohepatitis (NASH), defined as presence of hepatic steatosis, inflammation and hepatocyte ballooning, is increasing at an alarming rate in Australia and world-wide. While NASH is associated with the development of life-threatening diseases, including fibrosis, cirrhosis and liver cancer, there are currently no FDA-approved treatments for NASH and liver fibrosis. This gap has sparked significant investment in drug development for NASH, with >100 therapies currently in various phases of clinical trials. In addition, the frequent coexistence of NASH and type 2 diabetes has further intensified interest in devising comprehensive therapies to tackle both diseases simultaneously.

Our group's prior research identified Hexosaminidase A (HEXA) as a novel NASH-regulated hepatokine (i.e., liver-secreted protein) in both mice and humans. HEXA is an enzyme important in sphingolipid metabolism, converting ganglioside GM2 to GM3. These gangliosides are primarily found within lipid rafts, microdomains localized to the plasma membrane that are involved in cell signalling and nutrient transport. HEXA-induced remodelling of the lipid raft lipidome in liver and muscle is associated with reduced hepatic steatosis [1] and improved glycemic control through increased IGF1-mediated glucose uptake in skeletal muscle [2]. In addition, pilot data show that HEXA recombinant protein therapy in mice with hepatic steatosis reduces protein expression of proteins associated with inflammation and fibrosis, highlighting the potential for HEXA as a novel therapy for NASH and liver fibrosis.

Here we show that a long-lasting FC-HEXA protein analogue reduces cytokine release from Kupffer cells *in vitro*, the resident macrophages within the liver, and increases glucose metabolism in HepG2 liver cells. Similarly, using the MUP-uPA NASH mouse model (both wild-type (WT) mice with mild NASH and MUP-uPA mice with NASH and F2 fibrosis) we show that FC-HEXA treated mice have increased fatty acid oxidation in liver and glucose oxidation in both liver and skeletal muscle, which was associated with a significant improvement in hyperglycaemia, glucose tolerance and a trend to improved insulin sensitivity. However, contrary to our expectations, FC-HEXA treatment did not impact hepatic steatosis or fibrosis in both WT and MUP-uPA mice. Together, these outcomes suggest that HEXA might offer therapeutic benefits for type 2 diabetes but not NASH or liver fibrosis. Our experimental in vivo procedures did not utilize any anaesthetic, tranquilizing and muscle relaxant drugs

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The role of oestrogen in female skeletal muscle ageing: A systematic review

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Ageing is associated with a loss of skeletal muscle mass and function that negatively impacts the independence and quality of life of older individuals. Females demonstrate a distinct pattern of muscle ageing compared to males, potentially due to menopause where endogenous sex hormone production declines. This systematic review aims to investigate the current knowledge about the role of oestrogen in female skeletal muscle ageing. A systematic search of MEDLINE complete, Global Health, Embase, PubMed, SPORTDiscus, and CINHAL was completed from inception to 08/11/2022. The systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines and was registered in the international prospective register of systematic reviews (PROSPERO) (CRD42022374366). Studies were considered eligible if they compared a state of oestrogen deficiency (e.g. postmenopausal females) or supplementation (e.g. oestrogen replacement therapy) to normal oestrogen conditions (e.g. premenopausal females or no supplementation). Outcome variables of interest included measures of skeletal muscle mass, function, damage/repair, and energy metabolism. Quality assessment was completed with the relevant Johanna Briggs critical appraisal tool, and data were synthesised in a narrative manner. Thirty-two studies were included in the review. Nineteen studies (59%) had a low risk, 10 studies (31%) had a moderate risk, and three studies had a high risk (9%) of bias. Seventeen studies compared skeletal muscle outcomes in females across different menopausal stages. Overall, they showed that compared to premenopausal females, postmenopausal females display reduced muscle mass and strength, but the effect of menopause on markers of muscle damage and expression of the genes involved in metabolic signalling pathways remains unclear. Of 10 studies that investigated the effect of oestrogen supplementation, some suggest a beneficial effect of oestrogen replacement therapy on muscle size and strength, but evidence is largely conflicting and inconclusive, potentially due to large variations in the reporting and status of exposure and outcomes. The findings from this review points toward a potential negative effect of oestrogen deficiency in ageing skeletal muscle, but further mechanistic evidence is needed to clarify its role.

Urolithin A Reduces Markers of Inflammation and Muscle Damage, But Does Not Further Improve Endurance Running Performance in Well-Trained Males

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Urolithin A (UA) is a compound produced by gut bacteria following the consumption of food containing polyphenols such as pomegranates, cherries and berries. Clinical trials in elderly and middle-aged adults have demonstrated that supplementation with purified UA improves muscle strength, endurance, and biomarkers of mitochondrial health. These findings suggest UA may be an effective ergogenic aid to improve performance in endurance-trained athletes. To test this hypothesis, well-trained competitive male distance runners (*n*= 41, 27 ± 7 yr, $\dot{V}O_{2max}$ 66.4 ± 3.9 mL/kg/min) were recruited and randomised to consume either 1000 mg/d UA (*n*=22) or placebo (PL; *n*=19) for 4 weeks during an altitude training camp held at ~1700 m above sea level. Subjects completed tests for aerobic capacity ($\dot{V}O_{2max}$) and running economy at baseline and following the training camp. While at altitude, athletes performed a weekly downhill running protocol, with blood samples collected prior to exercise and at 1, 24, and 36 h post exercise for assessment of creatine kinase (CK) and C-reactive protein (CRP). Additionally, athletes completed either a 3000 m running time trial (n=10 PL, n=11 UA) or had skeletal muscle biopsies taken (n=9 PL, n=11 UA) pre- and post-camp to determine the impact of UA on performance and the regulation of skeletal muscle metabolism. Following 4 weeks of training, $\dot{V}O_{2max}$ increased in both groups, with no difference between UA and PL (5.0 ± 3.9% vs. 3.2 ± 5.4%, respectively, p=0.225). Gene ontology (GO) pathway analysis revealed that proteins associated with mitochondrial protein-containing complex, as well as organellar and mitochondrial ribosome were significantly upregulated in athletes consuming UA vs. PL (p<0.05), while proteins associated with immunoglobin complex were significantly downregulated (p<0.05). However, citrate synthase activity was not increased compared to baseline (p=0.130), and no changes were detected in maximal lipid (octanoylcarnitine, p<0.746) or carbohydrate (pyruvate, p=0.075) supported mitochondrial respiration in permeabilised muscle fibres in either group. Area under the curve (AUC) for CRP was decreased following downhill runs by week 3 in the UA group only, while CK was unchanged in both groups. Compared to baseline, 3000 m time trial performance improved following training in both PL (08:43 ± 00:15 vs. 08:54 ± 0:15 mm:ss, p<0.001) and UA (08:34 ± 00:17 vs. 08:46 ± 00:16 mm:ss, p<0.001), with no differences between groups (p=0.763). However, both the AUC for CK (p<0.05) and post-race rate of perceived exertion (p<0.05) were decreased in the UA group. Collectively, these results suggest that daily UA supplementation down-regulates markers of muscle damage and inflammation but does not improve endurance performance or mitochondrial function compared to placebo in well-trained male runners.

Overcoming mechanistic challenges to preserve muscle function in sarcopenia

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Sarcopenia was traditionally defined as an age-related loss of muscle mass, but more recent consensus statements from different international working groups, indicate sarcopenia being probable when low muscle strength or function is detected, and a diagnosis confirmed by the presence of low muscle quantity or quality. Thus, 'dynapenia', the loss of muscle strength not caused by neurological or muscular disease, may be the more relevant context for many older adults who do not present with a marked loss of muscle mass. These scenarios place greater emphasis on the need to better understand the mechanistic basis for age-related muscle weakness to develop therapeutic strategies able to preserve or improve muscle function, essential for maintaining independence and promoting healthspan in older adults.

Some of the important age-related impairments in muscle function include the progressive loss of strength and power, greater fatigue through a decline in muscular endurance, and a higher susceptibility to contraction-mediated muscle injury, especially as a consequence of sudden falls. All these muscle functional parameters can impact on an older person's independence and quality of life.

Despite the obvious benefits of exercise, particularly resistance training, in promoting healthy ageing, the reality is that physical activity (and nutrition) alone cannot stop the progressive and inevitable decline in muscle health. The ability to alter the course or trajectory of these age-related changes depends on understanding what underlies these deficits in muscle structure, function, and metabolism, and identifying pharmacotherapeutic targets.

Contributing mechanisms include (but are not limited to) changes in circulating muscle anabolic hormones and growth factors that maintain the balance between protein synthesis and protein degradation to maintain muscle fibre size; whether there is patent electrical contact between stimulating nerves and contracting muscle fibres; whether the local muscle or systemic environment is plagued by inflammation and/or oxidative stress; whether blood flow can be maintained to supply nutrients to contracting muscles; and age-related impairments in muscle regenerative capacity, attributed to some of the mechanisms already mentioned, and potentially to changes in the inherent properties of the muscle's resident population of satellite cells or muscle stem cells.

These potential contributing mechanisms identify as targets for pharmacotherapies and not addressing one or more of these factors could limit or compromise the efficacy of any intervening treatment. Aside from these mechanistic challenges, other barriers include the risk: benefit for different pharmacological interventions and the relative therapeutic window of opportunity for effectively addressing age-related physiological deficits (Lynch 2022).

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Sex-based difference in skeletal muscle of middle-aged and older adults in response to a period of disuse

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Onset of menopause in women occurs around the age of 50 and is characterized by the cessation of the endogenous production of the female sex hormones, estrogen and progesterone. The menopausal transition also increases women's vulnerability to the detrimental consequences of age-related muscle loss, sarcopenia, chronic disease, frailty and hospitalization in older women when compared to age-matched men (1, 2). Age-related decrements in muscle strength(3) and mass (4) are observed earlier in women than in men, around the age of menopause. While the mechanisms driving this menopause related decline in muscle health are yet to be fully elucidated but are likely related to action of estrogen in skeletal muscle. Serum estradiol is positively associated with greater muscle mass and strength in older women (5).

Periods of muscular disuse associated with illness and injury accelerate catabolism and compromise metabolic and muscle health, especially in aging adults. Findings from bed rest studies completed by our group identified key age and sex-specific responses to muscle disuse in middle-aged and older men and women. Unlike their age-matched, middle-aged, male counterparts, pre-menopausal women displayed a "more youthful", blunted catabolic response to seven days of bed rest. Conversely, older women (post-menopausal) appeared to have a greater propensity for a catabolic response to seven days of bed rest than their age-matched male counterparts.

The combination of longer life expectancy, repeated bouts of inactivity/illness and lower lean mass likely contribute to a higher risk for women to experience an accelerated decline in muscle health resulting higher rates of frailty and functional impairment, when compared to male counterparts(2, 6). Together these data indicate the necessity to consider sex-based differences in muscle metabolism, especially when conducting research in aging populations.

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Mitochondrial bioenergetics across the movement spectrum

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Mitochondrial aerobic metabolism is a highly regulated process that is rapidly activated during exercise to maintain metabolic homeostasis, a fundamental requirement for continuous muscle contraction to occur. Additionally, impaired mitochondrial biology has been directly linked with numerous pathological conditions, including skeletal muscle atrophy, insulin resistance, aging and cardiac dysfunction. Despite the importance of mitochondrial bioenergetics to cellular health, there remains an incomplete understanding of the mechanisms regulating oxidative phosphorylation, limiting our ability to identify therapeutic approaches to prevent numerous diseases. Within this context our team has focused on identifying mechanisms regulating mitochondrial membrane substrate transport, and leveraged this knowledge to elucidate nutritional approaches that preserve mitochondrial bioenergetics as a preventative medicine approach. In particular, we have focussed on identifying mechanisms regulating the rate limiting enzyme in the transport of lipids (carnitine palmitoyltransferase I: CPTI) and ADP/ATP (Adenine nucleotide translocase: ANT), investigated these mechanisms in diverse situations, and determined the ability of dietary nitrate to positively affect mitochondrial bioenergetics. This talk will summarize our recent findings on the regulation of CPTI and ANT during exercise, the development of insulin resistance, immobilization induced atrophy, aging, and sex differences. Moreover, we have recently uncovered that nitrate prevents high-fat diet-induced cardiac dysfunction, whole-body insulin resistance, dyslipidemia, hepatic dysfunction, skeletal muscle disuse-mediated reductions in mitochondrial protein synthesis rates (FSR) and prevented increased mitochondrial reactive oxygen species (ROS) emission in diverse metabolic situations. While these physiological outcomes are likely in part linked to the serial reduction of nitrate to systemic nitric oxide (NO)-mediated vasodilation, we have also utilized fecal microbial transplantation from nitrate-fed donors to prevent HFD-induced cardiac dysfunction in the absence of increasing serum nitrate or reducing blood pressure highlighting a gut-heart axis. Given these systemic, reproducible, and consistent effects, nitrate appears to represent a viable therapeutic approach to improve mitochondrial bioenergetics to combat compromised cardiometabolic health in diverse situations, which in part is related to how mitochondria respond to ADP.

Co-option of liver glycogen handling enzymes in the naked mole-rat heart

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As a subterranean eusocial mammal the naked mole-rat faces a particularly challenging environment characterised by patchily available food, low O_2 and high CO_2 levels. In response, naked mole-rats have evolved a suite of molecular and physiological adaptations which support its extreme longevity (37-year lifespan), extraordinary resistance to hypoxia and absence of cardiovascular disease. We have uncovered several metabolic adaptations which the naked mole-rat utilises to protect its tissues against extreme hypoxia and ischaemia. Naked mole-rats maintain large glycogen reserves in the heart at levels characteristic of liver where glycogen storage usually predominates. Under stressful conditions like ischaemia, naked mole-rat heart mobilises its vast glycogen stores to supply glucose and glycolytic intermediates to, amongst other processes, maintain sufficient ATP levels and protect the heart from ischaemia-related damage. Interestingly, although mouse heart utilises muscle-like glycogen metabolising enzymes to process its small glycogen stores, the naked mole-rat heart has borrowed glycogen-metabolising processes from the liver to more efficiently store and utilise glycogen. More surprisingly, naked mole-rats additionally use amylase, a starch metabolising enzyme in the intestine to efficiently breakdown glycogen and release glucose under ischaemia. This specialised cardiac glycogen utilisation system seems to be unique to the naked mole-rat and not found in closely related species of the African mole-rat genera. We have investigated the contribution of epigenetic remodelling to glycogen storage and breakdown in the heart of the naked mole-rat by investigating H3K27ac, H3K4me3 and H3K4me1 histone marks around glycogen relevant genes. We have found profound remodelling in the epigenetic landscape around these genes which promote a more adapted metabolism in the heart to deal with stresses associated with extreme states of energy and oxygen depletion.

Crosstalk between Inositol 1,4,5 trisphosphate receptors ($InsP_3R$) and Ryanodine Receptors (RyR) contributes to disrupted Ca^{2+} handling and arrhythmogenic activity in human heart failure.

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Disruption in intracellular Ca^{2+} handling mechanism underlies the reduced contractility and the greater propensity for arrhythmogenic activity and sudden cardiac death associated with heart failure (HF). Highly organised rhythmic increases in intracellular [Ca^{2+}] are central to cardiomyocyte function coupling electrical depolarization of the sarcolemma with contraction through the process of excitation contraction coupling (ECC). Fundamental to this process is the activation of Ca^{2+} release from the sarcoplasmic reticulum Ca^{2+} store via ryanodine receptor channels (RyRs) by Ca^{2+} entering the cell through L-type voltage gated channels on the sarcolemma. This Ca^{2+} induced Ca^{2+} release (CICR) occurs at specialized compartments at which the sarcolemma and the underlying SR come into close proximity known as dyads, which are distributed throughout the cell volume owing to regular invaginations of the sarcolemma known as T-tubules. In disease, T-tubule density is reduced leading to decreased coupling between cell depolarization and Ca^{2+} release via RyR. Consequently, cell contraction is reduced and arrhythmogenic propensity increased. We have shown that inositol 1,4,5, trisphosphate receptors ($InsP_3R$) Ca^{2+} channels are also expressed in cardiomyocytes and release Ca^{2+} in response to $InsP_3$ generated downstream of neurohormones such as endothelin-1 and angiotensin. We show that $InsP_3R$ expression and activity is increased in HF human cardiomyocytes resulting in lower amplitude Ca^{2+} transients and arrhythmogenic activity, which we observe at both cell and tissue level, including in human. This action of $InsP_3Rs$ requires Ca^{2+} dependent crosstalk with RyRs, which we functionally demonstrate under conditions of RyR inhibition. Supporting this finding, we show using super resolution imaging overlap of $InsP_3R$ and RyR and by mathematical modelling, increased RyR activity when co-localised with $InsP_3Rs$. Together our data show an important role for $InsP_3Rs$, and Ca^{2+} release from them, in the diminished function and increased likelihood of fatal arrhythmias in human HF.

Manipulating muscle growth to improve dystrophic pathology in mouse models of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the *DMD* gene and aberrant expression of the dystrophin protein, leading to progressive muscle wasting and weakness. To date, there is still no cure or effective treatment. Given the genetic basis of this disease, gene-based interventions will likely eventually lead to a cure, but in the interim there is an urgent and unmet need for strategies that can improve quality of life and prolong lifespan so that patients can receive perfected gene therapies. Formoterol, an FDA-approved β_2-adrenoceptor agonist, used as a bronchodilator in the management of respiratory diseases, has re-emerged as a clinical intervention to regulate skeletal muscle mass (Kalsen *et al*., 2016). It has potential to ameliorate DMD pathology by improving muscle mass and strength (Harcourt *et al*., 2007). To this end, we investigated whether formoterol could improve the pathophysiology of muscular dystrophy in two well-characterised murine models of DMD.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). We used dystrophin deficient *mdx* mice that have a mild dystrophic pathology and *dko* mice (deficient in dystrophin and utrophin) that exhibit a more severe phenotype like that in DMD patients. Four-week-old male wild type (C57BL/10), *mdx*, and *dko* mice were administered saline (control, *i.p.*), or formoterol (100 or 1000 µg/kg/day, *i.p.*) for 4 weeks. At the end of the treatment period, all animals were anaesthetised with sodium pentobarbitone (60 mg/kg, *i.p.*) for assessment of contractile properties of tibialis anterior (TA) muscles *in situ*. Mice were then killed by cardiac excision while anaesthetised deeply.

Formoterol administration increased TA, gastrocnemius, rectus femoris and plantaris muscle mass of wild type and *mdx* mice (relative to tibia length) ($P < 0.05$), confirming the muscle anabolic effects of formoterol. Interestingly, formoterol treatment reduced the cumulative force deficit with lengthening contractions in a dose-dependent manner relative to saline treated mice for both *mdx* and *dko* mice ($P < 0.05$).

The findings indicate that TA muscles from dystrophic mice were less susceptible to contraction-induced injury after formoterol treatment, in both the mild and more severe mouse models of DMD. Future studies will assess the long-term effects of formoterol to investigate the potential of anabolic stimuli to ameliorate the dystrophic pathophysiology.

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Post-Developmental Disruption of Muscle PolG1 Exonuclease Activity Induces Mitochondrial Stress and a Cachexia-like Phenotype

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Mitochondrial DNA (mtDNA) is replicated within the mitochondria by DNA polymerase gamma (PolG), which has three enzymatic actions: polymerase activity, exonuclease activity (proofreading), and base excision repair. As the sole polymerase found in mitochondria, PolG is essential for the replication and maintenance of mtDNA, and subsequently, mitochondrial health and function. Mutations to the PolG gene can lead to impaired mtDNA replication and/or sequential accumulation of mtDNA mutations/deletions, which can ultimately result in mitochondrial dysfunction. The existing mouse model used for the study of PolG driven mitochondrial dysfunction, known as the PolG "mutator" mouse ($PolG^{D257A}$), is a global transgenic with systemic symptoms that can confound experimental findings. Consequently, this provides multiple challenges for investigating PolG mediated defects in a specific tissue. Here, we describe a floxed-PolG mutator mouse ($PolG^{fl/fl}$-mutator), generated by inserting LoxP sites flanking the proofreading/repair exonuclease domain of the PolG gene. We have subsequently crossed $PolG^{fl/fl}$-mutator with inducible-Cre mice (ACTA1-Cre-ERT2) to generate mature mice with muscle specific-PolG insufficiency. CTX injections were performed under an inhaled anaesthetic (isoflurane: induction 5%, maintenance 1-2%) and euthanasia performed using a mix of Ketamine/Xylazine (200/40mg) that was injected IP. Muscle specific-$PolG^{fl/fl}$-mutator mice began losing weight approximately 15-weeks post-tamoxifen, accompanied by elevated FGF21 and GDF15, but with no alterations in food intake, glucose or insulin tolerance. Proteomic and functional analysis demonstrated reductions in CI, CIII and CIV abundance and activity. Combined with transcriptomics, our data indicates initiation of the integrated mitochondrial stress and unfolded protein response including alterations to ribosomal and translational machinery, consequently leading to mitochondrial dysfunction. Thus, our tissue specific PolG mutator model both recapitulates aspects of the traditional mutator mouse whilst displaying unique characteristics, highlighting the advantages of investigating mtDNA mutation-driven pathology in a tissue specific manner.

Modulating Membrane Lipids as Therapeutics for Cardiovascular Disease

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Plasmalogens are a class of phospholipids that are abundant in tissues like the brain, muscles, heart and certain immune cell types. Lower circulating and tissue plasmalogen levels have been associated neurological, muscular and more recently, cardiometabolic disorders including atherosclerosis, coronary artery disease and heart failure. With the increasing prevalence and economic burden of cardiovascular disease, plasmalogens present as an attractive therapeutic target as their levels can be artificially modulated via dietary supplementation of their metabolic precursors, alkylglycerols (AKG). Previous studies have demonstrated that AKG supplementation can provide therapeutic beneficial in a variety of disease settings, and studies have shown this supplementation strategy to be effective in settings of cardiovascular dysregulation. My research program now aims to elucidate the protective effects of plasmalogen modulation using preclinical models of cardiovascular disease and to develop new delivery methodologies to improve translatability of this therapeutic strategy to patients with cardiovascular disease.

Redistribution of calcium content in slow-twitch skeletal muscle fibres mediated by SR Ca^{2+} leak

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Muscle contraction depends on tightly regulated Ca^{2+} cycling between intracellular stores. Aberrant Ca^{2+} leak through ryanodine receptor 1 (RyR1) on the sarcoplasmic reticulum (SR) membrane causes a spectrum of effects, including susceptibility to heatstroke and malignant hyperthermia (MH), as well as severe muscle weakness (Dianese et al., 2009; Lopez et al., 2018). Our recently published article (Lamboley et al., 2021) revealed that, in mouse fast-twitch (FT) muscle fibres, RyR1 Ca^{2+} leak triggered a precise redistribution of Ca^{2+} across the SR, cytoplasm and mitochondria in FT fibres by altering the Ca^{2+} permeability of the tubular (t-) system membrane. The impact of RyR1 leak-mediated Ca^{2+} redistribution was to reduce the amount of Ca^{2+} release required for normal contraction and to increase the resting metabolic rate (Meizoso-Huesca et al 2022, PNAS). Because slow-twitch (ST) differ to FT fibres in SR and mitochondrial abundance, Ca^{2+} handling capacity and fatiguability, we chose to investigate the effect of accumulative *RYR1* gain-of-function mutation in ST fibres.
RYR1 knock in (KI) mice colony established at The University of Queensland were used in this study. Wild type (WT), heterozygous (HET) RyR1 KI, and Homozygous (HOM) *RYR1* KI mice were euthanized via CO_2 overdose and soleus muscles were rapidly excised. Individual fibre segments from those muscles were mechanically skinned under paraffin oil so that they still maintained their endogenous Ca^{2+} content. Prior to skinning, some fibres were exposed to a physiological solution containing 1 mM rhod-5N and allowed sufficient time to equilibrate inside the t-system. Skinned fibres with t-system-trapped rhod-5N were imaged on a confocal microscope to assess RyR Ca^{2+} leak (Lamboley et al 2021). Fibres attached to the force transducer allowed measurement of the total amount of endogenous, SR and mitochondrial Ca^{2+} contained (Fryer & Stephenson, 1996; Lamboley et al., 2021). Additionally, we attached soleus muscles to a force transducer to determine twitch and tetanic force response in each genotype.
Twitch and tetanic force responses were not different between WT and HET soleus muscle. HOM soleus muscle showed a raised resting tension and reduced peak force compared to WT muscles. A gene-dose effect of *RYR1* mutation on RyR Ca^{2+} leak was observed in skinned fibres. Consistent with the increased leak, SR Ca^{2+} content reduced and mitochondrial Ca^{2+} content increased in a stepwise fashion. Additionally, sex-specific differences were observed, with males showing results consistent with a greater leak compared with females. Comparisons to FT fibres from the same mice showed ST fibres are leakier than FT. Across FT and ST fibres, SR Ca^{2+} content was greater in FT than ST fibres and this pattern was maintained across genotypes. Mitochondrial Ca^{2+} content consistently increased with decreasing SR Ca^{2+} content across the genotypes.
Our results are consistent with fibre Ca^{2+} redistribution supporting the generation of normal force responses with the increased RyR Ca^{2+} leak in HET muscle. Our results predict a higher cytoplasmic Ca^{2+} content in ST than FT fibres. This is consistent with the lower Ca^{2+} release of ST fibres than FT to support force generation and a higher basal turnover of ATP to maintain SR Ca^{2+} content which can be supported by a larger mitochondrial content of ST compared to FT fibres.
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Understanding the NMJ's defects in cancer cachexia

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Most patients with advanced solid cancers exhibit features of cachexia, a debilitating syndrome characterized by significant and progressive loss of skeletal muscle mass and strength that ultimately accounts for 20-30% of cancer deaths. The underlying mechanisms of this multifactorial syndrome are incompletely defined and no effective therapies are available. Muscle mass loss and muscle function impairment are two important clinical aspects of cancer patients, being associated with poor outcomes. Preservation of muscle mass despite unchanged tumor growth, fat loss and pro-inflammatory cytokine production improves survival in mice (1). The BMP (Bone and Morphogenetic Protein) axis plays a critical role in muscle mass regulation (2, 3). The BMP pathway is also known to regulate neuromuscular junction (NMJ) development and plasticity in Drosophila but its role in controlling muscle-nerve synapse and NMJ remodeling in adult mammals, particularly in pathological contexts, is poorly investigated. Through morphological observations, molecular analyses, and electromyography recordings, we revealed an unexpected effect of tumor burden on NMJ structure and function in a preclinical model of cancer cachexia* (4). Cancer changes the transcriptome of the muscle and, particularly, it triggers the expression of Noggin (an extracellular BMPs antagonist) in the muscle which blocks the trophic actions of BMPs not only on muscle fibres but also on the nerve, causing muscle wasting, NMJ dismantling and loss of myofibers innervation. Increased Noggin expression is sufficient to induce dismantling of the neuromuscular junction and muscle fiber denervation. Moreover, morphological and molecular analyses of skeletal muscle biopsies from pre-cachetic and cachetic patients affected by pancreatic, colorectal and esophageal cancer were performed and compared. In the same patients the serum levels of markers of NMJ remodelling were evaluated. Our findings present important new insight into pathogenic mechanisms of muscle wasting and weakness in cancer cachexia and new treatment strategies to restore a functional musculature in a cancer type-specific manner.

* Surgical procedures were performed under inhalation of isoflurane in medical oxygen with post-operative carprofen or meloxicam analgesia

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Association Between Muscle-Specific Detrimental Phenotype in Dysferlin-Deficient Mice and Altered Calcium Handling

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Dysferlin is a membrane protein located in the transverse tubules of skeletal muscle fibers, where it participates in processes of membrane repair, structure maintenance and calcium handling. Deficiency of this protein is causative for Limb Girdle Muscular Dystrophy type 2B and Miyoshi Myopathy (Argov et al., 2000). Interestingly, Dysferlinnull fibres display signatures of ryanodine receptor 1 (RyR1) dysregulation, including a reduction of voltage-induced calcium release, as well as alterations of RyR1s clustering (Lukyanenko, et al., 2017; Barefield et al., 2021). RyR1 dysfunction has been associated with different myopathies, but the role of RyR1 dysfunction in the aetiology and progression of Dysferlin deficiency-associated myopathies remains elusive. Here, we employed mechanically skinned muscle fibres to study the role of Dysferlin on calcium handling in skeletal muscle. To study a potential correlation between calcium mishandling and disease progression, we analysed calcium handling properties in muscle fibres from a muscle that presents a severe dystrophic phenotype (Psoas) and one that displays a mild pathological presentation (Tibialis Anterior) from the Dysferlin-deficient BlaJ mouse and compared them to their respective WT controls. This was done at two different ages, corresponding to before (8 weeks) and after (36 weeks) the dystrophic phenotype was evident. Interestingly, psoas fibres showed greater RyR1 calcium leak compared to fibres from the TA in the wild-type (WT) control groups. This difference was exacerbated in muscles from BlaJ mice. Concomitant to the progression of the dystrophy, a greater RyR1 Ca2+ leak was observed in the psoas muscle (comparing 2 vs 9 months old), accompanied by a chronic activation of store-operated calcium entry (SOCE). In addition, PMCA-mediated Ca2+ extrusion and SOCE upon acute SR Ca2+ depletion were impaired in BlaJ psoas fibres compared to their WT counterpart. These changes in calcium handling were associated with differences in intracellular Ca2+ distribution, where a higher mitochondrial calcium content was observed in those groups with greater RyR1 leak. Together, this data shows that inherent RyR1 leak properties exist between Psoas and TA fibers and that this difference becomes exacerbated in absence of Dysferlin. Additionally, absence of Dysferlin not only results in RyR1 Ca2+ leak exacerbation but also in a muscle-specific hindering of Ca2+ handling mechanisms such as Ca2+ extrusion via PMCA and acute SOCE.

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Genome-wide CRISPR screen identifies CBX4 as a novel regulator of hepatic lipid metabolism

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver condition in developed countries. NAFLD involves a spectrum of liver diseases that range from simple steatosis to its progressive forms, non-alcoholic steatohepatitis (NASH) and liver fibrosis. NAFLD is characterized by defective lipid metabolism, including changes in fatty acid uptake and oxidation, lipoprotein secretion and *de novo* lipogenesis, which causes hepatic steatosis and inflammation, key events that precede and contribute to fibrosis and are important for disease progression. Hence, tackling early metabolic defects (i.e., inhibiting hepatic steatosis) is likely to prevent the development of NASH and metabolic co-morbidities.

To systematically uncover genes required for hepatic lipid accumulation, we performed five independent, pooled genome-wide screens using a GeCKO library targeting 19,050 known human genes and 1864 miRNAs. HepG2 cells were transduced and screened after 4 days of puromycin selection, to exclude un-transduced cells and sgRNAs targeting essential genes. Cells were treated with or without fatty acids (in the presence of BSA), followed by Bodipy lipid staining and fluorescence-activated cell sorting (FACS). Side scatter (SSC) was used as a secondary measure of increased lipid droplet content. In BSA-treated control cells, the highest 10% 'Bodipy plus SSC' were collected to select mutants that retained high levels of lipid due to defects in lipid processing ('negative regulators'). In the FA-treated cells, we collected the lowest 10% 'Bodipy plus SSC', thereby screening for cells bearing mutations to genes necessary for FA-induced lipid accumulation ('positive regulators').

This led to the generation of two ranked lists of genes required for lipid processing/metabolism: 946 potential negative regulators and 734 positive regulators. Using single sgRNA knockout, we validated the top 36 novel targets using FACS and ^{14}C metabolic tracing, and identified Chromobox 4 (CBX4) as a novel regulator of hepatic lipid metabolism. Deletion of CBX4 in HepG2 cells significantly reduced fatty acid uptake and lipid storage, while simultaneously increasing mitochondrial fatty acid oxidation, which was associated with an overall reduction of lipid accumulation in cells. Future studies are aimed at investigating if deletion of CBX4 in the liver may confer protective benefits in NAFLD.

Chronic stress abrogates the beneficial effects of exercise in murine models of breast cancer

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Introduction:
Chronic stress can promote the development and progression of cancer. One major stressor, namely social isolation, has been linked to a worse prognosis in multiple cancers, including breast cancer (1). Separately, exercise has been found to have stress-relieving effects (2). While there is substantial evidence reporting the benefits of exercise on the development and progression of breast cancer, the potential of exercise to counteract the adverse effects of social isolation on tumour progression remains unknown.

Methods:
To investigate this, two breast cancer models, 4T1.2.luc and the transgenic polyoma middle T oncoprotein mouse model of luminal breast cancer (MMTV-PyMT), were employed. Mice were either single-housed or housed with one other littermate (dual-housed) and given access to either functional (exercise) or locked (sedentary) running wheels from 7 to 13 weeks of age. Primary tumour volume and lung/lymph node metastases were measured twice a week for the duration of the study.

Results:
Social isolation did not impact running wheel activity or body weight. Primary tumour volume was reduced ($P<0.05$) when dual-housed mice exercised, however there was no difference between exercise and sedentary mice when single-housed. Exercise appeared to reduce metastasis recorded at experimental endpoint for both single and dual housed mice. As social isolation is a known stressor, plasma corticosterone levels were measured in both single and dual housed mice to determine whether this may have influenced the benefits of exercise on primary tumour progression, however, no differences were observed.

Discussion:
Together, these studies indicate that exercise is beneficial for delaying tumour progression in the mouse models of breast cancer, but only when mice are not housed alone. Experiments are currently ongoing to investigate the mechanisms by which social isolation overrides the benefit of exercise training on tumour progression.

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Cardiac adipose: the good, the bad and the unknown

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Visceral adipose depots are highly active endocrine organs, releasing factors into the blood that can deleteriously influence other organs. Given its immediate proximity to the heart, interest in pericardial visceral adipose as a determinant of cardiac risk has rapidly emerged. Pericardial adipose tissue describes the combined epicardial and paracardial adipose depots surrounding the heart. It has important physiological functions in regulating myocardial fatty acid availability, but excess adiposity is a cardiovascular liability. A focus of our lab has been to investigate the strong link between cardiac adiposity and atrial fibrillation. Studies show that both infiltration of adipose into the epicardial surface of the atria and release of pro-fibrotic paracrine factors exert a physical barrier to normal atrial conduction pathways. This leads to regional areas of slowed conduction across cardiomyocytes that can disrupt normal conduction pathways and introduce 're-entrant' activity – repetitive circular electrical pathways that are critical to the onset and maintenance of atrial fibrillation. Our published findings have moved the field forward in a new direction, demonstrating a novel inter-cellular communication axis within the heart between pericardial adipose and neighbouring cardiomyocytes. We showed that this communication axis conveys the paracrine actions of infiltrating pericardial adipose, causing localised structural and electrical remodelling of adjacent cardiomyocytes and promoting the conduction heterogeneity that can culminate in atrial fibrillation. We now extend these findings to further investigate the molecular mechanisms underlying adipose-cardiomyocyte communication and its role in the development of heart disease.

Imbalances in fatty acid during pregnancy and outcomes for her child's health

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Fatty acids are essential for normal cellular function and in addition, are critical for normal fetal growth and development. Essential fatty acids can only be obtained in the diet. Linoleic acid (LA), an n-6 polyunsaturated essential fatty acid (PUFA), is required in low concentrations for normal cellular function, but proinflammatory at high concentrations. The consumption of LA has increased in recent years due to availability in processed foods. Thus, women of reproductive age are also consuming elevated concentrations of LA.

Using a rodent model, we have investigated the effect of a maternal diet high in LA (HLA) content on pregnancy, placental and offspring developmental outcomes. Further, we have investigated whether restoration of the postnatal diet to a control low LA (LLA) concentration can improve any adverse developmental outcomes. In our model female rats were fed low LA (LLA; 1.44% energy from LA: recommended daily intake) or HLA (6.21% energy from LA: what Australians are consuming) diets for 10 weeks before pregnancy, and during gestation/lactation. One cohort were sacrificed just before birth (embryonic day (E20). Offspring were weaned at postnatal day 25 (PN25), fed LLA or HLA diets and sacrificed at either postnatal day (PN) 40 or PN180 (6 months old).

The main findings from these studies were that a HLA diet alters maternal and offspring circulating fatty acids at E20, PN40 and PN180. At E20, HLA diet reduced the number of male offspring, altered placental fatty acids and reduced maternal leptin (leptin is a key modulator of development). At PN40, maternal HLA altered offspring fatty acid concentrations, cardiac function, hepatic function and circulating lipids in a sex specific manner. Further, at PN180, maternal HLA altered fatty acids and lipid metabolism in a sex specific manner, and increased leptin in females. Thus a maternal HLA diet may alter placental function and program cardiac dysfunction, metabolic disease and alter lipid metabolism in the adult offspring.

Skeletal Muscle Redox Balance in Aged Conditions

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Ageing is a part of normal life cycle and is associated with the progressive decline in cellular function and the deterioration of physiological processes. The precise mechanisms underlying this inevitable progressive physiological decline remain unclear, but they are thought to involve the diminishing capacity of organisms to adapt to stressors, such as oxidants, namely reactive oxygen species (ROS). Reactive oxygen species (ROS) are continuously generated in skeletal muscle during exercise, instigating adaptive responses that promote metabolic health. However, the precise mechanisms by which exercise, and ROS promote metabolic health remain unclear. Both mitochondria and NADPH oxidases (NOXs) have been implicated as sources of ROS in muscle. Our studies have shown that skeletal muscle NADPH oxidase 4 (NOX4) is primarily responsible for exercise induced H_2O_2 generation, preventing the oxidative damage that otherwise contributes to the development of insulin resistance. In the current study, we demonstrate that skeletal muscle NOX4 deficiency exacerbates the decline in muscle function and insulin sensitivity, highlighting that the age-associated decline in NOX4 levels accelerates the onset of frailty. Our findings point towards skeletal muscle redox homeostasis being instrumental for metabolic health and healthy ageing.

Repairing muscle without stem cells after exercise

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Movement is natural to us. From slowly crawling out of bed to the occasional fast to catch the bus, our muscles are hardwired to mechanically comply to our command. It is therefore easy to overlook the extent of stress and strain they endure as a biological system. And despite an architecture geared for contraction, our muscles are prone to ripping and tearing. Thankfully, skeletal muscles possess an outstanding capacity to repair injuries using stem cells (satellite cells) which activate, proliferate and either create brand new muscle cells (myofibers) or patch up existing ones. However, is satellite cell dependent regeneration appropriate for all types of muscle injury? In this work, we identify a new mechanism of myofiber intrinsic repair. After exercise-induced damage, myonuclei express repair genes and migrate to the site of injury to locally deliver mRNA for cellular reconstruction. In contrast to stem cell-dependent regeneration, myofiber self-repair is fast, innate, and tailored to minor injuries. Myofiber self-repair represents a new perspective from which to understand muscle regeneration in muscle disorders, aging or exercise.

The long non-Coding RNA OIP5-AS1 is Sufficient but Not Necessary for Skeletal Muscle Differentiation in Murine Models

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Introduction: Skeletal muscle diseases, such as sarcopenia and Duchene muscular dystrophy, result in a severely compromised quality of life and even death. Unfortunately, there are still very limited therapeutic options available. Long non-coding RNAs (lncRNAs; RNA transcripts >200 nucleotides in length that do not encode proteins) have recently emerged as novel therapeutic targets to improve skeletal muscle regeneration due to their role as critical regulators of gene programs under physiological and pathological conditions. Our group identified a lncRNA termed OIP5-AS1 that is highly enriched in skeletal muscle and is shown to be upregulated during myogenesis. We thus sought to test whether manipulating OIP5-AS1 expression could impact on skeletal muscle differentiation and development.

Methods/Results: To demonstrate a functional role for OIP5-AS1 in muscle differentiation *in vitro*, we used various methods to manipulate the expression of OIP5-AS1 in C2C12 cultures. Using an adenovirus expressing shRNA against OIP5-AS1 in C2C12 myoblasts, we observed a significant impairment in myotube formation and a reduction in the mRNA expression of myogenic genes. Conversely, increasing OIP5-AS1 expression, using different viral transduction methods resulted in increased myotube formation and earlier expression of myogenic genes, suggesting that increasing the expression of OIP5-AS1 is sufficient to promote enhanced myotube fusion. To investigate if OIP5-AS1 is necessary for muscle regeneration *in vivo*, we tested the muscle regenerative response in two different mouse models of OIP5-AS1 knockout (KO), including a global KO mouse and an inducible, muscle specific mouse model (OIP5-AS1 floxed crossed with iACTA1-cre ERT2). At 8 weeks old, these mice were injected with cardiotoxin (CTX, 10 μM) into one of their *Tibialis anterior* (TA) and *Extensor digitorum longus* (EDL) muscles, which induced complete ablation of mature muscle fibres and thus a controlled regeneration model. As an internal control, saline was injected into the other TA and EDL muscles of the same mice. Muscles were harvested at day 1, 3, 7, 14 and 28 post-CTX injection to assess the effect of OIP5-AS1 KO versus wildtype in different stages of myogenesis. Inconsistent with the role of OIP5-AS1 over-expression in promoting muscle differentiation, there was no major impairment in muscle regeneration in either mouse model of OIP5-AS1 KO compared to their wildtype littermates.

Conclusion: Overall**,** our study demonstrated that overexpression of OIP5-AS1 enhances muscle differentiation *in vitro*, but its deletion has minimal impact on muscle regeneration *in vivo*. This suggests that OIP5-AS1 may be sufficient to promote accelerated muscle differentiation, but may not be necessary for muscle regeneration, at least within the models and conditions tested here, and warrants further assessment to fully elucidate OIP5-AS1's function and potential therapeutic applications.

Intrinsic muscle stem cell dysfunction in Akita mice, a model of Type 1 diabetes

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Type 1 diabetes mellitus (T1DM) is a systemic metabolic disease characterised by an autoimmune response against pancreatic beta cells, leading to impaired insulin secretion and hyperglycaemia. Patients with T1DM exhibit reduced muscle force, an increased susceptibility to muscle injury, and impaired muscle regeneration (Orlando *et al*., 2017, Dial *et al*., 2021). Given the critical role of muscle stem cells (MuSCs) for muscle regeneration and maintenance of muscle health, we tested the hypothesis that MuSC function is compromised in Ins2 (Akita) mice, a murine model of T1DM.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8^{th} ed. Canberra: NHMRC). Akita mice contain a heterozygous mutation in the *Ins2* gene, causing beta cell stress and development of diabetes at 4 weeks of age. Akita mice were compared with wildtype (WT) littermate controls not containing the *Ins2* mutation. Mice were anaesthetised during intramuscular injections using vaporized isoflurane (4-5% induction, 1-2% maintenance, in 80% oxygen) and tibialis anterior (TA) muscles of the right hindlimb were injected with barium chloride ($BaCl_2$, 1.2% w/v, in saline) to cause muscle damage. For repeated injuries, mice were allowed to regenerate for 28 days before the next intramuscular injection, performed up to three times. Dapagliflozin was added to the drinking water of WT and Akita mice at 5 weeks of age to reduce hyperglycaemia through excretion of glucose in the urine. At endpoint, mice were killed by rapid cervical dislocation and the hindlimb skeletal muscles carefully excised for isolation of MuSCs or stored for later immunohistological analyses.

Reduced myofiber size and absolute muscle force was observed in the TA of Akita mice after three consecutive muscle injuries compared to WT animals. Histological and flow cytometry analysis revealed a reduction in MuSC number in Akita mice after 8 weeks of diabetes. MuSCs from Akita mice exhibited signs of aberrant activation in the absence of injury, with a higher proportion of Ki67+ cells in TA muscle cross-sections and upregulated gene sets associated with proliferation in proteomic analysis of freshly isolated MuSCs. Isolated MuSCs from 12-week-old Akita mice formed myotubes with reduced diameter, lower basal respiration and impaired force output in 3D culture when cultured at physiological glucose levels. Treatment of diabetes with dapagliflozin ameliorated the loss of MuSC number, aberrant activation, and reduced myotube force and size *in vitro*.

These data suggest that in T1DM, the impaired muscle force generating capacity after injury may be attributed to a reduction in MuSC number and compromised hypertrophy of myotubes following MuSC differentiation. This reduced MuSC population may be due to disruption of the quiescent state by a hostile diabetic environment. Our study suggests that deficits in MuSC-derived myotube hypertrophy persist after removal from the diabetic environment, but dysfunction can be rescued by early treatment of hyperglycaemia with dapagliflozin. Follow-up studies will assess whether early maintenance of glucose control can prevent MuSC dysfunction and improve muscle regeneration.

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Mitochondrial dynamics and gene expression are impaired in ALS patient derived iPSC-derived lower motor neurons

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder causing skeletal muscle atrophy and respiratory failure due to motor neuron death. ALS destroys cells and tissues that are highly dependent on healthy mitochondria; organelles which facilitate the production of ATP. Mitochondria are highly dynamic, and constantly undergo fusion and fission to maintain a healthy network that is crucial for sustaining cellular functions. Our study aims to investigate the extent of damage to the mitochondrial network and mitochondrial dynamics in ALS using human induced pluripotent stem cell-lower motor neurons (hiPSC-LMNs). In this study we generated hiPSC-LMNs from four healthy individuals and four patients with ALS (1 sporadic, 1 ALS fronto-temporal dementia, 1 *C9orf72*, 1 S*OD1*^{D77Y}). We also generated a mutant CRISPR-Cas9 *TARDBP*^{A382T} iPSCs from a healthy control line. All ALS hiPSC-LMNs exhibit a motor neuron disease like phenotype, with severe motor neuron death and/or TDP-43 mislocalisation by day 35 *in vitro*. Live-cell imaging revealed mitochondrial network fragmentation in ALS hiPSC-LMNs. On average, ALS patient derived hiPSC-LMNs mitochondria demonstrated more fission:fusion events, are more punctate in nature ($p<0.001$), have shorter branch lengths ($p<0.001$), less branches ($p<0.001$), and less branch junctions ($p<0.001$). Gene expression array data demonstrates significant impairment in complex I and V ($p<0.01$), with aberrant regulation across complex III and IV. This study is the first to demonstrate mitochondrial network fragmentation in ALS hiPSC-LMNs, and paves the way for future therapeutic targeting and understanding of patient specific disease mechanisms.

Exercise, but not glucocorticoids, modifies circulating levels of lipocalin-2 and its forms in young males

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Objectives
Elevated lipocalin-2 (LCN2) is associated with increased risk of cardio-metabolic disease. LCN2 has different forms including a polyaminated (hLCN2), secreted predominantly by osteoblasts, and non-polyaminated (C87A and R81E), secreted predominantly by adipocytes. Glucocorticoids negatively affect bone and energy metabolism while exercise improves energy metabolism. As such, both glucocorticoids and exercise potentially regulate circulating LCN2. We hypothesised that glucocorticoids would suppress LCN2 and its forms, at baseline and following exercise
Methods
In a double-blind, randomised crossover design, nine young, healthy males (aged 27.8 ± 4.9 years, BMI 24.4 ± 2.4 kg/m^2) completed 30 mins of high intensity aerobic exercise (4 sets x 4 mins at 90-95% HRR) after glucocorticoid (20 mg prednisolone) or placebo treatments. Blood was collected at baseline, immediately post-exercise, 1 h post-exercise (variant analyses only), and 3 h post-exercise. LCN2 was analysed using commercially available ELISA (LCN2 Abcam – primary outcome) and different forms of LCN2 (hLCN2, C87A and R81E) using in-house assays previously validated, secondary outcomes.
Results
LCN2 (Abcam) was elevated after prednisolone compared with placebo (main treatment effect of ~10%; $p = 0.015$). Prednisolone treatment had no effect on individual LCN2 forms (all $p > 0.53$). Regardless of treatment, or assay used, LCN2, C87A, R81E, and hLCN2 increased immediately after exercise (all $p < 0.033$). LCN2, but not the forms, remained elevated at 3 h post-ex ($p = 0.048$).

Conclusion
In contrast to our hypothesis, prednisolone had a limited effect on LCN2, however, both LCN2 and its forms are transiently increased by acute exercise in young healthy males, independent of assay used. The role of LCN2 and it forms in exercise and glucose metabolism warrant further investigation.

Table 1. The effects of acute aerobic exercise and prednisolone treatment on circulating LCN2

	ebo Treatment				**lnisolone Treatment**			
	line	·ex.	ost-ex.	ost-ex.	line	·ex.	ost-ex.	ost-ex.
2 nL)Abcam	± 26	± 28*	)ata	± 32*	± 27#	± 35#*	)ata	± 35#*
N2 (ng/mL	± 67	± 67*	± 48	± 51	± 44	± 42*	± 48	± 55
A (ng/mL)	30	25*	14	15	7.6	16*	17	15
E (ng/mL)	44	64*	49	60	32	66*	38	55

Mean ± SD. * $p < 0.05$ compared to baseline; # $p < 0.05$ compared to placebo (main treatment effect).

Hypochlorous acid exposure impairs skeletal muscle function and Ca^{2+} signalling: implications for Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a fatal X-linked genetic disease characterised by severe wasting of skeletal muscle. The mechanisms underlying the DMD pathology likely involve the complex interaction between inflammation, oxidative stress and impaired Ca^{2+} handling however the link between these factors is currently unclear. Hypochlorous acid (HOCl) may form a link between these factors. HOCl is a highly reactive oxidant produced endogenously via myeloperoxidase; an enzyme secreted by neutrophils that is significantly elevated in dystrophic mdx mouse muscle (Terrill et al., 2013). Oxidation of Ca^{2+} handling proteins by HOCl may chronically increase resting Ca^{2+} concentration. This study hence aimed to determine the effects of HOCl on skeletal muscle function and its potential contribution to the dystrophic pathology.

Extensor digitorum longus (EDL) and interosseous muscles were surgically isolated from anesthetised C57 (wild-type) and mdx (dystrophic) mice for measurement of *ex vivo* force production and intracellular Ca^{2+} concentration respectively. Mice were anaesthetised via an intraperitoneal injection of sodium pentobarbitone (40 mg/kg body weight, Lethabarb, Virbac, Australia) before removal of EDL and interosseous muscles. Mice were then euthanised using an overdose of sodium pentobarbitone (> 120 mg/kg of body weight, intraperitoneal injection). For experimentation using EDL muscle, isolated whole EDL muscles were selectively exposed to 200 μM HOCl during an 80 minute contractile protocol in an *ex vivo* skeletal muscle test system. Experimentation was subsequently performed using the same protocol but with either i) 150 μM of the ryanodine receptor (RyR) blocker, tetracaine; or ii) 300 μM of the TRP channel blocker, streptomycin. For Ca^{2+} imaging experimentation, single fibres were chemically isolated from whole interosseous muscles and loaded with the Ca^{2+} fluorescent dye, FURA-2 AM. The effects of 10 μM HOCl on resting Ca^{2+} concentration and Ca^{2+} transient amplitude was subsequently investigated with no blocker present, 150 μM tetracaine or 300 μM Gd^{3+} (blocker of TRP channels). All data were analysed via two-way ANOVA with Tukey post-hoc analysis.

In whole EDL muscle, HOCl (200 μM) significantly decreased maximal specific force and increased resting tension in both C57 and mdx muscles (p's < 0.0001). Both tetracaine and streptomycin lessened the effects of HOCl on resting tension (tetracaine - C57: $p = 0.0001$, mdx: $p = 0.0361$; streptomycin – C57: $p = 0.0037$, mdx: $p = 0.0272$) while streptomycin lessened the effects of HOCl on maximal force also (C57: $p = 0.0014$, mdx: $p = 0.0115$). In single interosseous fibres, HOCl (10 μM) increased resting Ca^{2+} (C57: $p = 0.015$, mdx: $p = 0.011$) and decreased Ca^{2+} transient amplitude (C57: $p = 0.024$, mdx: $p = 0.017$). Similar to whole EDL muscle, these effects of HOCl could be ameliorated with either tetracaine (resting Ca^{2+} only, C57: $p = 0.0045$, mdx: $p = 0.0017$) or Gd^{3+} (resting Ca^{2+}, C57: $p = 0.0037$, mdx: $p = 0.0272$; Ca^{2+} transient amplitude, C57: $p = 0.0297$, mdx: $p = 0.0233$).

These results demonstrate the potent effects of the endogenously relevant oxidant, HOCl on skeletal muscle contractile function which may be due to HOCl-induced oxidation to RyR and TRP channels causing impaired Ca^{2+} signalling. Hence, HOCl may provide a link between chronic inflammation, oxidative stress and impaired Ca^{2+} signalling that is characteristic of DMD and thus presents a potential target for therapeutic treatments for DMD.

An Atlas for Ageing Methylome

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During ageing, the human methylome exhibits both differential (i.e. change in mean) and variable (i.e. change in variance) shifts, along with a general rise in entropy. However, it remains unclear whether DNA methylation sites that increasingly diverge between people (i.e. variably methylated positions (VMPs)) are distinct from those undergoing changes in mean methylation levels (i.e. differentially methylated positions (DMPs)), which changes drive entropy, how they contribute to epigenetic age measured by epigenetic clocks, and whether cell type heterogeneity plays a role in these alterations. To address these questions, we conducted a comprehensive analysis using > 32,000 human blood methylomes from 56 datasets (age range = 6-101 years). Our findings revealed an unprecedented proportion of the blood methylome that is differentially methylated with age (48% DMPs; FDR< 0.005) and variably methylated with age (37% VMPs; FDR< 0.005), with many sites overlapping between the two groups (59% of DMPs are VMPs). We observed that bivalent and Polycomb regions become increasingly methylated and divergent between individuals, while quiescent regions lose methylation in a more homogeneous manner between individuals. Unexpectedly, both chronological and biological clocks, but not pace-of-aging clocks, show a strong enrichment for those CpGs that accrue both mean and variance changes during aging. Furthermore, we uncovered that it is the accumulation of DMPs shifting towards a methylation fraction of 50% that drive the increase in entropy, resulting in an overall smoothening of the epigenetic landscape. However, approximately a quarter of DMPs oppose this direction of change, exhibiting anti-entropic effects. While DMPs were mostly unaffected by changes in cell type composition, VMPs and entropy measurements showed moderate sensitivity to such alterations. This investigation represents the largest to date of genome-wide DNA methylation changes and ageing in a single tissue, offering valuable insights into primary molecular changes that hold meaning for chronological and biological ageing.

Affinity purification-mass spectrometry and single fibre proteomics reveals mechanistic insights of C18ORF25

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The beneficial outcomes of exercise are typically achieved through a series of protein phosphorylation networks initiated by engaging in physical activity. Recently, we performed phosphoproteomic analysis of human skeletal muscles subject to endurance, sprint, and resistance exercise to identify novel regulators of exercise and metabolic health. This revealed phosphorylation of S67 on C18ORF25 upregulated across all three exercise modalities, suggesting a fundamental role in exercise signalling.

Subsequent investigation validated S67 on C18ORF25 as a substrate of AMPK, a known master regulator of metabolism. A whole-body C18ORrf25 KO mouse model revealed abnormal body composition and reduced skeletal muscle function, specifically in fast twitch fibres. Furthermore, proteomic as well as contraction-induced phosphoproteomic analysis of KO skeletal muscles revealed impaired PKA signalling and attenuated phosphorylation of SR calcium handling and contractile proteins. While a general understanding of phenotypic outcomes and regulated pathways downstream of C18ORF25 have been established, we have yet to determine its direct mechanistic actions.

To identify interacting partners of C18ORF25 and those regulated by AMPK-dependent phosphorylation of S67, we performed anti-FLAG affinity purification coupled to mass spectrometry (AP-MS) of differentiated C2C12 myotubes expressing either GFP, FLAG-C18ORF25-WT or FLAG-C18ORF25-S66/67A (phospho-dead) that were either untreated as controls (C) or dual treated with the AMPK activators AICAR and A-769662 (AA). This revealed an enrichment of proteins associated with endocytosis, regulation of the actin cytoskeleton, ribosomal proteins, mitophagy and lipid proteins as well as Ras and Rap1 signalling pathways in WT vs GFP. Analysis of protein:protein associations that display differential enrichment to wild-type C18ORF25 in response to AMPK activation revealed an interconnected network of GTPases. Interestingly, the protein with the greatest differential binding to C18ORF25 in response to AMPK activation and lost following mutation of S66/67A was GNAS, required for cAMP-dependent PKA signalling.

We also performed phenotyping followed by single cell proteomics of single fibres isolated from WT vs KO soleus muscles. This revealed significant proteomic remodelling in fast twitch fibres of KO mice only, including differential regulation of PKA substrates and known calcium handling proteins, such as CAMK2g and AHNAK. We also correlated all measured phenotypes to each quantified protein in a pairwise manner which identified a total of 752 significant protein: phenotype associations. This revealed several novel associations with uncharacterized proteins such as a positive correlation between PROB1, enriched in skeletal muscle, with pCa50 and negative correlation of PPIB with specific force where PPIB is an SR-localised chaperone implicated in musculoskeletal health. Taken together, our data provide unique insights into the function of C18ORF25 and its role in skeletal muscle physiology.

All NASH-HCC models are wrong, but some are more useful than others.

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Background: Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal cancers worldwide. Originally arising from chronic liver disease such as hepatitis B and C virus, HCC prevalence is now increasingly ascribed to the obesity pandemic (1). With no available effective drugs, it is imperative to further understand the mechanisms that drive disease progression. This hinges on the development of representative clinical *in vivo* models, which are either based on dietary interventions (high fat diet, Western diet high in cholesterol, fructose diet), the introduction of a carcinogen (CCl_4, diethylnitrosamine), or genetic modifications (Diamond model, *MUP-uPA* model) (2). To date, the *MUP-uPA* mouse model, when fed a high-fat diet (HFD), is one of the only models that truly displays all disease characteristics (3). However, like other transgenic and dietary mouse models of NASH-driven HCC, the *MUP-uPA* + HFD model is time consuming. Developing a mouse model of accelerated HCC, whilst still showing all disease characteristics, will greatly help the research field.

Methods: 6-week-old male *MUP-uPA* and wild type (WT) mice were either fed a high-fat diet (HFD) for 34 weeks or a modified Western diet (MWD), a Western diet where all glucose is substituted for fructose, for 18 weeks. At 24 weeks of age, mice underwent a liver biopsy surgery to obtain a small piece of liver tissue, which was used for hematoxylin and eosin staining. The liver was visually observed for tumour incidence before surgery recovery. Mice on the HFD were aged a further 16 weeks and humanely sacrificed at 40 weeks of age and tissues collected.

Results: At 24 weeks of age, none of the WT (n=22) or *MUP-uPA* (n=20) mice fed a HFD displayed tumours. Tumour incidence increased to 6/20 in the *MUP-uPA* + HFD group, whilst remaining absent in the WT + HFD group at 40 weeks of age. At 24 weeks of age, *MUP-uPA* (n=24) mice fed a MWD displayed adenomas (n=6) and fulminant HCC (n=8), respectively, mimicking tumour incidence present at 40 weeks of age when *MUP-uPA* mice were fed a HFD.

Conclusion: Feeding a MWD to *MUP-uPA* mice accelerates NASH-induced HCC development and might represent a more time-efficient mouse model to study disease progression.

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Mapping the muscle molecular ageing process in females: a lifespan approach

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Sex as a biological variable has been significantly overlooked in physiology and muscle research, which historically has focused on male participants. Skeletal muscle, the largest tissue of the body, is one of the main tissues underpinning sex-based differences. At baseline, it has up to 3000 genes that are differentially expressed in males and females, contributing to numerous sex-specific phenotypic traits, for example in muscle growth, regenerative capacity, and fibre type distribution. It follows that our understanding of skeletal muscle biology cannot be assumed from single- or combined-sex study designs. A relevant example is the ageing trajectory of female skeletal muscle and its functional outcomes, which do not mirror and therefore cannot be inferred from male-specific knowledge.

In humans, the metabolic environment driving age-related muscle loss is sex-specific and age-dependant. Specifically, the loss of ovarian follicular activity and altered hormonal status accompanying menopause are associated with increased risk of age-related muscle loss in females when compared to age-matched males. Identifying the fundamental sex-specific mechanisms underlying age-related muscle loss is therefore essential for all research that seeks to explore the pathophysiology of ageing.

Existing studies on the sex-specific effects of ageing often focus on specific events of the female life cycle (such as pregnancy or menopause) or dichotomise large population groups (such as pre-menopausal versus post- menopausal females). Such study designs are more feasible and provide necessary information to advance the field but lead to systematic underestimation of the extent of variation in outcomes between groups. For example, ageing individuals close to but on opposite sides of the pre-determined cut point are characterised as being very different rather than, in fact, being very similar.

This study aims to map the functional, cellular, molecular and epigenetic processes underpinning the regulation of female skeletal muscle mass across a continuum of ageing. A combination of muscle physiology, cell physiology, -omics and advanced bioinformatics approaches are used to integrate these processes and investigate their associations with sex hormone levels across each decade of adulthood. Inference of biological pathway activity can then be drawn from the multiple levels of evidence (functional, hormonal, proteome, transcriptome, epigenome), providing the most accurate foundation to date to establish cause-and-effect relationships between molecular regulators and age- and sex-specific phenotypes.

Examination of Mitochondrial calcium uniporter complex proteins in the *mdx* mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive and debilitating muscle wastage disease which is characterised by unsustainable, and ultimately fatal, cycles of fibre necrosis and regeneration. DMD fibre damage arises partially from extracellular calcium (Ca^{2+}) influx, a consequence of the functional absence of dystrophin, a key structural protein in the cell membrane (sarcolemma) of muscle fibres. In healthy skeletal muscle fibres, Ca^{2+} released from the sarcoplasmic reticulum (SR) into the cytosol activates contractile machinery and relaxation occurs when the cytosolic [Ca^{2+}] returns to resting levels, primarily via active SR reuptake. A fraction of the released SR Ca^{2+} enters mitochondria and contributes to oxidative phosphorylation, thereby assisting with the high ATP demands of the contractile machinery and cytosolic Ca^{2+} regulation (1). In DMD, increased mitochondrial [Ca^{2+}] uptake may contribute to observed mitochondrial dysfunctions, including reduced ATP output and increased reactive oxygen species production, aberrations which have been suggested to perpetuate damage and impede regeneration (2). The mitochondrial Ca^{2+} uniporter (MCU) complex is the main channel for Ca^{2+} import across the inner mitochondrial membrane. Several proteins comprise and regulate the MCU complex including; the pore forming MCU, the dominant negative MCUb, a controversial regulator MCUR1 and gate keeping MICU1 homodimers (MICU1-1d) or heterodimers with MICU2 (MICU1-2d), of which the MICU1-1d has a lower Ca^{2+} threshold for activation than MICU1-2d (1, 3). It is not known whether the abundance of MCU complex proteins changes in dystrophic muscle.

The *mdx* mouse is a model of DMD wherein muscle damage peaks at ~28 days of age before dystrophin loss is compensated for by the upregulation of the homologue, utrophin. At ~70 days, an age by which the skeletal muscle of wildtype (WT) mice is mature, *mdx* muscle is relatively stable, as the reduced damage allows for improved regenerative outcomes (4). WT (C57/BL10ScSn) and *mdx* (C57BL/10ScSn-*Dmd*^{mdx}) mice aged 28- and 70-days (n=5) were anaesthetised via intraperitoneal injection (10 µl/g, Nembutal) prior to dissection and culling by cardiac incision (AEC 12-31, 13-48) (5). Protein abundance was determined in whole muscle homogenates of tibialis anterior via calibrated Western blotting. All data were tested for statical significance by one-way ANOVA, Holm-Šídák's post hoc analysis. Cytochrome c oxidase subunit IV (COXIV) abundance was analysed to indicate mitochondrial content and no significant differences were found between test groups. Increased abundance of MCU and MICU1-1d, was observed in WT28 compared to WT70 and was not seen in *mdx*28 compared to *mdx*70 (3.5-fold and 2.6-fold respectively, $p<0.05$). A higher abundance of MCU and MCUb was observed in *mdx* compared to WT at 70, but not 28 days (3.2-fold, $p<0.05$ and 1.6-fold, $p<0.001$ respectively). Additionally, the abundance of MCUb and MICU1-2d was higher in *mdx*70 than it was in *mdx*28 (1.4-fold, $p<0.001$ and 1.6-fold, $p<0.01$ respectively).

These data show significant changes in the abundance of proteins regulating mitochondrial Ca^{2+} import, in both WT and *mdx* mice, that highlight possible links between MCU complex activity and muscle maturation. This study provides a basis for future mechanistic studies in muscle maturation and may offer direction for future medical interventions aimed at promoting fibre maturation, and therby muscle function, in individuals with DMD.

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Characterising the role of UFMylation in skeletal muscle

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Ubiquitin Fold Modifier-1 (UFM1) is a ubiquitin-like modifier that plays an important role in regulating metazoan biology. It shares structural similarity to ubiquitin and uses a 3-enzyme pathway cascade (E1, UBA5; E2, UFC1; and E3, UFL1) to attach itself to substrate lysine residues for post-translational modification of target proteins. UFSP2 (deUFMylase) is the UFM1 specific protease and this collective process, referred to as UFMylation, appears enriched at the endoplasmic reticulum. Although the chemistry of UFM1 attachment to target proteins is well known, its physiological function and role in cellular processes remains poorly understood.

To this end, a recent study (1) identified RPL26 (a ribosomal protein) as the primary target of UFMylation in cell culture. More recently, we (2) provided the first evidence demonstrating that UFMylation is a novel regulator of skeletal muscle function. However, many questions remain unanswered such as what are the *in vivo* substrates of UFMylation and is UFMylation regulated in pathophysiological conditions? Herein, we report our findings on the development of a novel antibody-based enrichment method to identify UFMylation sites by proteomics and further quantify the *in vivo* UFMylome during skeletal muscle atrophy in humans.

All mouse experiments were approved by The University of Melbourne Animal Ethics Committee and mice were anaesthetised with isoflurane (4% in oxygen, 1 L/min) for tissue isolation. Collection of human vastus lateralis muscle biopsies, including the informed signed consent of participants, was in accordance with procedures approved by The University of Queensland.

To identify UFMylation sites in skeletal muscle, proteins were extracted in denaturing buffers and digested with trypsin thereby leaving a remnant valine-glycine (VG) attached to the substrate lysine of UFMylated proteins via an isopeptide bond (VG-ε-K isopeptide). Using 40 mg of protein lysate from mouse gastrocnemius muscle, together with pooled anti-VG-ε-K antibody clones from Cell Signaling Technologies, we identified 236 UFMylation sites following immunoprecipitation of enriched peptides. UFMylated proteins were associated with cellular compartment terms such as the Z-disc, sarcomere, myosin filament, and sarcoplasmic reticulum. Investigation of known protein:protein interactions revealed extensive UFMylation of sarcoplasmic reticulum and contractile apparatus related proteins such as calsequestrin, RYR, SERCA, and myosin, including the known UFMylation site K134 on RPL26.

Western blotting of skeletal muscle biopsies from human Amytrophic Lateral Sclerosis (ALS) subjects showed a significant increase in UFMylated substrates as compared to control subjects. Using our novel antibody-based enrichment strategy to identify UFMylation sites by proteomics, we quantified 96 unique VG sites on 39 proteins, including myosin heavy chain (MHC) I, in ALS subjects. This prompted us to perform a more detailed investigation of myosin UFMylation using purified myosin from mouse gastrocnemius muscle. Remnant UFMylation peptides were identified for all major myosin isoforms (MHC I, IIa, IIb, IId) with UFMylation sites enriched in the head region.

Given the critical role of myosin in force generation, the above results suggest a previously unreported functional role for UFMylation in skeletal muscle contraction. We next plan to manipulate the UFMylation pathway in skeletal

muscle via genetic and enzymatic manipulation, and then use single muscle fibres to further investigate this post-translational modification process.

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A Hypothalamic circuit regulates immunity in accordance with whole-body energy balance.

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Background: Adaptive immunity is essential to host survival, but poses significant energetic costs associated with lymphocyte proliferation and cytokine production. Accordingly, converging evidence has highlighted that adaptive immunity is subject to metabolic regulation. Long term dietary restriction induces the repartitioning of lymphocyte populations across immune organs. However, the mechanisms underpinning the metabolic regulation of immunity are currently unclear, particularly in the context of short-term fasting. A key player in the central regulation of metabolism is the hypothalamus. The arcuate nucleus of the hypothalamus (ARC) represents a crucial nexus for the translation of peripheral metabolic signals into whole body metabolic adaptations. In the fasted state, ARC neurons respond to such signals through modulating autonomic nerves, producing profound changes in whole body energy expenditure. Despite this, no research to date has examined the role of the ARC in the regulation of fasting immunity.

Results: We first aimed to identify the effects of fasting on the partitioning of lymphocyte populations across key immune organs. We used immunofluorescence techniques to quantify T cells ($CD3^+$) and B cells ($B220^+$) in spleen, thymus, and bone marrow extracted from C57BL/6J mice that were either fasted for 20 hours or provided *ad libitum* access to food. Fasted mice displayed increased T- and B-cells in the thymic corticomedullary junction (CMJ; $p = 0.024$, $n = 8$) and bone marrow, ($p = 0.013$, $n = 10$) respectively, while both cell types were reduced in the splenic white pulp (T cells, $p = 0.005$; B cells, $p < 0.001$, $n = 10$). We next aimed to quantify changes in the level and activity of innervation to key immune organs in the fasted state. We used immunofluorescence to quantify sympathetic innervation (TH^+) in key immune organs from fasted and fed mice. We show that fasting promotes increased sympathetic innervation of the thymus ($p = 0.003$, $n = 12$), but not the spleen or bone marrow. We next used a transgenic mouse model to label and quantify active innervation within key immune organs in fasted and fed states. We demonstrate that fasting results in the potentiation of neuronal activity in the spleen ($p < 0.001$, $n = 8$), and its suppression in the bone marrow ($p = 0.046$, $n = 11$). Finally, we sought to determine the capacity of ARC fasting circuits to regulate immune partitioning. We found that chemogenetic activation of fasting responsive neurons in the ARC ($n = 14$) of *ad libitum* fed mice significantly reduced T cells in the thymic CMJ ($p = 0.021$) and spleen ($p = 0.007$), and increased B cells in the spleen ($p < 0.001$) and bone marrow ($p = 0.0014$), when compared with control mice.

Conclusions: The results of this study bolster a growing body of evidence that adaptive immunity is subject to metabolic regulation and add to current literature through the identification of a novel brain–immune axis contributing to this metabolic regulation.

Creating immersive learning environments in higher education

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Leveraging 21st-century technologies through the creation of virtual galleries we're able to shift the paradigm of medical science education. This innovative approach not only addresses digital literacy but also fosters collaboration, creativity, and innovation among students. This is particularly evident in the realm of online education, where recent advancements in world-building applications and AI interfaces have significantly enhanced distribution and accessibility.

Moreover, the integration of gamification into educational platforms has shown to be a transformative element, elevating student attentiveness, engagement, and interaction with course materials. Collaborating closely with academics and professionals, immersive and interactive simulations are tailored for student cohorts. Leveraging cutting-edge tools such as 3D scanners, 360 cameras, Virtual Reality, and 3D modelling software like Blender, alongside modern game engines like Unity, users can create digital twins of anatomical structures, immersive 360-degree videography, and surgical simulations.

These technologies extend the learning experience to both flatscreen and head-mounted displays, providing heightened accessibility and nuance for students. The use of 3D modelling and Virtual Reality enables students to explore and comprehend educational content at varying levels of detail, ranging from a micro to macro scale. Notably, recent strides in AI have introduced conversational agents that allow students to engage in dynamic dialogues, offering realistic scenarios with varied responses. This not only enriches the learning experience but also lightens the workload for educators.

In this presentation, we will showcase compelling use cases that highlight the seamless integration of these technologies into education, emphasizing their capacity to transform the landscape of medical science education and nurture a generation of digitally literate and creatively engaged students.