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Mollie Holman Lecture

Developing and refining a blended 1st year anatomy and physiology course with embedded studio-based brief lecture videos

Andrew Moorhouse[1,2], Christina Byun[3], Nathan Colquhoun[4], Matt Oxley[4]

[1]School of Medical Sciences, University of Sydney, Sydney, Australia
[2]Department of Physiology, UNSW Sydney, Australia
[3]Department of Anatomy, UNSW Sydney, Australia
[4]Media Unit, Faculty of Medicine and Health, UNSW Sydney, Australia

University students are frequently choosing the flexibility to watch recorded lectures online, as opposed to face-to-face attendance. Educators therefore have the challenge to deliver bespoke online lectures in an engaging and interactive format. We therefore implemented a blended learning approach when developing a new first year undergraduate introductory Anatomy and Physiology course, where lectures were delivered as online learning packages followed by face-to-face practical classes and active-learning tutorials. In 2023 (311 students) tutorials focussed on clinical contexts, while in 2024 (402 students) content was reduced and tutorials were focused more on revision activities. Students were from diverse backgrounds in one of five Health Professional programs (Physiotherapy, Exercise Physiology, Nutrition, Pharmacy and International Public Health). Online asynchronous learning packages were built using Articulate Rise360 software and consisted of lecturettes embedded as brief (≈10 mins) videos aligned to each learning outcome, and supplemented with clinical case introductions, relevant text and images, formative quizzes, and a final learning aims checklist. Some video snippets were professionally produced and edited in the studio, enabling improved audio and video quality, and enabling analogies and concepts to be presented in a more dynamic way.

An end of course survey was undertaken across both years and the effects of course revisions on grades and overall satisfaction were quantified. Overall grades increased by 4.8%, from a mean of 71 in 2023 (n=311) to 76 in 2024 (n=402). The proportion of students that agreed or strongly agreed with the statement, “overall I was satisfied with the quality of the course”, increased from 76% in 2023 (n=154) to 93% in 2024 (n=261). In 2024, two specific questions regarding delivery style were added to the end of course 5-point Likert survey. 90% of students agreed or strongly agreed that the asynchronous learning packages were an effective way to learn weekly content, while 93% agreed or strongly agreed that the studio-based lecture videos made the learning activity more engaging and effective.

Developing fit-for-purpose online asynchronous learning activities using studio based lecture videos and interactive activities require more time and resources than traditional lectures, however, can be an effective and engaging way to deliver content that meets students’ desire for flexibility. When combined with in-person revision and practical classes, we conclude that it is an effective way to engage diverse early-year students in learning biomedical science. Such blended learning approaches may support the development of students towards effective careers as health professionals.

UNSW Human Research Ethics approval (#iRECS9166)

Abstract: EF1

Human Ai-ssessments

Michael Leung

Monash University

As artificial intelligence (AI) continues to reshape both academic and professional landscapes, educators have a responsibility to prepare graduates to use emerging digital tools and strengthen their digital literacy[1]. A growing body of literature demonstrates that securing un-invigilated assessments is increasingly difficult, if not impossible[2]. In response, some educators have attempted to address AI use by returning to campus based, sit-down examinations. However, this approach directly opposes the recent movement toward authentic assessment, which aimed to reduce reliance on traditional exams in universities.

The present study aimed to design two take home assessments that encouraged collaboration between student and AI. The goal was to help students develop the skills and knowledge needed to navigate an AI-enabled world[1].

For the first assessment, students were asked to use AI to confirm one of six assigned glucose metabolism pathways. They were then required to interact with AI to transform this information into three potential teaching activities for a second-year university practical class. From these, students selected one idea to fully develop for marking.

For the second assessment, students were provided with a GraphPad Prism file investigating university students' responses to different stressors. They were tasked with extracting all significant within- and between-group effects from a two-way ANOVA with Sidak's post-hoc test. These results were then used to prompt AI to generate both a written abstract and a suggested design for a graphical abstract. Students were required to create a final graphical abstract based on these suggestions.

For assessment 1, the average mark was 77.8% (SD ± 9%, n = 530). Initial survey feedback indicated mixed experiences: some students reported unclear instructions and anxiety about using AI, while others appreciated the opportunity to be creative within a science unit. Notably, some students did not enjoy generating graphics and figures. One of our key aims in this task was to encourage students to use AI for repetitive and drafting tasks, freeing up more time for creativity and critical thinking. On average, students reported spending ~4.5 hours on this assessment.

On reflection, several challenges were identified, including issues with clarity of instructions, marking consistency, and alignment with assessment goals. These areas will be refined in future iterations. Quantitative data for assessment 2 are still being collected (assessment due 13th September).

References:

[1] Digital health curriculum framework

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Abstract: EF2

Chatting to our Students' Future Selves: Using AI Chatbots to Explore Career Pathways in First-Year Medical Science Education

Craig R Campbell, Adam Ghanem, Mohamad Elzein, William Havellas, Claudio Corvalan Diaz Philip Poronnik, and Martin Brown.

School of Medical Sciences, The University of Sydney, NSW.

Artificial Intelligence (AI) is changing the future of work. What will the future that we're preparing our students for look like? What skills must we ensure our students have to be able to work in that future?

Integrating AI into higher education presents exciting opportunities to enhance student engagement, reflection, and career planning. In a novel assessment for a first-year Medical Science unit, students created an AI chatbot representing themselves ten years into the future. The chatbot described their envisioned career, how the role may evolve, and the skills and achievements needed to succeed.

Rather than requiring programming expertise, students used plain English to build their chatbot personas, leveraging natural language as a new form of digital literacy. This approach democratised access to AI tools and emphasised clear communication and conceptual thinking. Students learned how chatbots work and were challenged to express their ideas precisely so their chatbot could respond meaningfully to questions about their future professional identity.

The assessment served multiple pedagogical purposes. First, it introduced students to emerging digital technologies in a hands-on, creative way. Second, it prompted deep reflection on their motivations for enrolling in a Medical Science degree and their long-term career aspirations. Third, it encouraged exploration of the impact of AI on biomedical and health-related professions, including current technological capabilities and future projections. Finally, it required students to identify and articulate the technical and interpersonal skills that they would need to develop to thrive in their chosen fields.

Preliminary analysis of student submissions revealed diverse imagined futures, including clinical roles, biomedical engineering, research careers, and entrepreneurial ventures. Many students demonstrated insight into how AI might reshape their professions, citing literature on automation, diagnostic tools, and personalised medicine. Others reflected on the enduring importance of human-centred skills such as empathy, communication, and ethical reasoning.

This assessment fostered career awareness and digital fluency, and aligns with broader educational goals that include critical thinking, self-directed learning, and adaptability. It provided a platform for students to envision their professional trajectories in a rapidly changing world and consider how their current studies could serve as a foundation for future success.

By sharing this initiative, I aim to highlight the potential of AI-based reflective assessment and practical tasks to enrich physiology and medical science education.

Abstract: EF3

Twelve Tips for Navigating the Frontier of AI-assisted assessments: Challenges, Skills, and Solutions

Suzanne Estaphan[1], David Kramer[2], and Harry J Witchel[3]

[1] School of Medicine and Psychology, College of Science and Medicine, Australian National University, Canberra, Australia.
[2] Graduate School of Medicine, University of Wollongong, Wollongong, Australia.
[3] Brighton and Sussex Medical School, University of Sussex Campus, Brighton, United Kingdom.

Background: The rise of artificial intelligence (AI) is transforming educational practices, particularly in assessment. While AI may support the students in idea generation and summarization of source materials, it also introduces challenges related to content validity, academic integrity, and the development of critical thinking skills. Educators need strategies to navigate these complexities and maintain rigorous, ethical assessments that promote higher-order cognitive skills.

Aims: This manuscript provides practical guidance for educators on designing take-home assessments (e.g. research-based assignments) in the AI era. It explores how AI can be leveraged to enhance student learning during these assessment tasks and offers educators the ability to develop personalized feedback while maintaining academic integrity and avoiding potential risks to students' skill development.

Description: Twelve tips are presented, organized into four key areas: (1) Co-developing AI literacy among students and educators, (2) designing assessments that prioritize process over output, (3) validating learning through AI-free assessments, and (4) preparing students for AI-enhanced workplaces by developing AI communication skills and promoting human-AI collaboration. These strategies emphasize ethical AI use, personalized feedback, and creativity.

Methodology: This guidance was developed through a collaborative, consensus-driven process involving a consortium of three educators with diverse academic backgrounds, career stages, and perspectives on AI in education. Members, holding experience in higher education across the UK, USA, Australia, and MENA regions, brought varied insights into AI's role in education. The team engaged in an iterative process of refining recommendations through bi-weekly virtual meetings and offline discussions, grounded in collaborative research and action research methodologies. This approach ensured the development of a comprehensive, practical, and context-sensitive framework for AI use in assessment.

Conclusion: By adopting these approaches, educators can balance the benefits and risks of AI in assessments, fostering authentic learning while preparing students for the challenges of an AI-driven world. This framework ensures that AI supports, rather than undermines, the integrity of educational outcomes.

Abstract: EF4

Integrating Planetary Health in an undergraduate Exercise Physiology assessment using co-design

Marwa Abdoul Ghayour*[1], Liza Barbour[2], Christopher Thompson[3] & Joanne Caldwell[1,3]

[1]Department of Physiology, Monash University. [2]Department of Nutrition, Dietetics & Food, Monash University. [3]Biomedicine Discovery Institute, Faculty of Medicine, Nursing and Health Sciences, Monash University

Introduction: The triple planetary crisis of climate change, pollution and biodiversity loss is threatening the health of current and future generations (United Nations, 2024). Our future health workforce is well placed to promote planetary health; however, they are currently underprepared to address the complex, contemporary challenges our global population is facing. This two-year project was designed to simultaneously build the capacity of educators and empower students by facilitating the co-design of planetary health curricula. This study had two aims: to understand the extent to which integrating planetary health education into an Exercise Physiology unit using curriculum co-design is effective at improving knowledge, attitudes and self-efficacy amongst student and to identify the planetary health concepts featured in student responses to a co-designed assessment activity and examine how they relate to the Sustainable Development Goals (SDGs).

Methods: To achieve these aims, five stages of research were conducted to develop, pilot and evaluate the new assessment activity, stage 1 involved the initial workshop with Planetary Health Education Champions (1 Educator and 3 Students) from an exercise physiology unit to conceptualise and develop the assessment activity. Stage 2 involved piloting the new assessment activity with students enrolled in the unit during 2023 (n = 240), including a five-question pre-poll questionnaire to measure knowledge, attitudes and self-efficacy. Stage 3 involved qualitative data analysis of submitted assessment responses from students. Data were then deductively analysed using the Sustainable Development Goals as the thematic framework. Stage 4 involved analysis of pre-post poll data to explore the efficacy of the activity using unpaired samples t-test (Table 1). Stage 5 involved facilitation of a post-pilot co-design workshop with the same Planetary Health Education Champions to refine the assessment activity for future use. Insights from the pre- and post-poll data, assessment responses, and reflections from Champion students and their educator, highlighting what worked well and what could be improved, were used to guide the refinement process.

Results: One of the assessment activities required students to answer the question: Based on your research topic and your desire to improve planetary health, what is one call to action for the government, your peers and/ or educators? Thematic analysis of student responses to this question revealed that physiology students' work was relevant to seven of the 17 Sustainable Development Goals. Example student work attributable to two of the SDGs: Goal 4 (Quality education): "The government must place a compulsory teaching at university, schools and other educational institutions about planetary health, what it is and how we as a society can contribute in improving the planetary health" and goal 13 (Climate action): "One call to action for the government is that governments should conduct investigation… and make relevant laws or policies to ensure that agricultural food is more eco-sustainable, eco-friendly, reduce dependence on fossil fuels."

Table 1. Pre-post student poll prompts

Student responses before (n = 169) and after (n = 152) exposure to the planetary health assignment demonstrated 5 out of the 5 prompts demonstrated a statistically significant improvement (Table 1).

Pre-Post Student Poll Prompts	p-value
I know how to best explain what planetary health means for healthcare professionals.	≤ 0.0001
I know what healthcare professionals in my field can do to address the causes and consequences of climate change.	≤ 0.0001
Planetary health should be core business for healthcare professionals.	≤ 0.01
I feel equipped to advocate for change to promote planetary health within my field.	≤ 0.0001
My training has prepared me to address the causes and consequences of climate change within my field.	≤ 0.0001

Conclusion: Integrating planetary health curricula into tertiary education can effectively improve knowledge, attitudes and self-efficacy amongst students. Applying a planetary health lens to existing assessment tasks can prompt students to relate their work to the Sustainable Development Goals (SDGs). To adequately equip our future workforce, planetary health education must be integrated into the curriculum, and this must occur urgently as the triple planetary crisis continues to escalate.

Reference: United Nations Climate Change. What is Triple Planetary Crisis. https://unfccc.int/news/what-is-the-triple-planetary-crisis Accessed 16th, September 2024.

Abstract: EF7

Academic Spaces as Sites of Belonging: How First-Year Anatomy and Physiology Students Connect with Others.

Louise Ainscough and Hainian Yu

School of Biomedical Sciences, University of Queensland, St Lucia Qld 4072, Australia

Belonging is a fundamental human need, with the desire to form strong, stable and positive interpersonal relationships shaping our cognitions, emotions and behaviours (Baumeister & Leary, 1995). Within the university context, a student's sense of belonging can be shaped by their connections with peers, staff, and their institution (Allen et al., 2024). A strong sense of belonging has been associated with improved academic outcomes, such as increased engagement, motivation and retention (Allen et al., 2024). This study examined how first-year students connect with others as an indicator of belonging.

Participants were enrolled in a first-year anatomy and physiology course for nursing and midwifery students in semester 1 of 2023. As part of a reflective assessment task, students answered the question "Universities are communities, where you can connect with others, including peers, teaching staff, mentors and future employers. How have you connected with others during this semester?" Responses from consenting students (n=123) were analysed using inductive thematic analysis (Braun and Clarke, 2006).

Most students (93%) reported making connections with others during the semester, with only 7 students stating that they had not made connections. On average, students reported making connections in 2 ± 0.2 different ways. Most students reported making connections in academic settings (82%), particularly through placement (27%), active learning classes (27%), study groups (23%) and lectures (21%). A smaller proportion of students reported making connections during co-curricular or social events (18%), such as through student associations (8%). One-quarter of students reported connecting with others in ways that could be classified as both academic or social, such as through social media (15%) or the library (6%). A multiple linear regression demonstrated that student demographics (degree, gender, international status and age) did not impact on the number of ways students reported connecting with others ($p = 0.14$). In addition, student retention was not influenced by how many different ways they reported connecting with each other, although the results approached significance (One way ANOVA; $p = 0.06$).

Our study suggests that students are most likely to connect with others in academic settings that provide small group active learning experiences. As such, curricula should be designed to include ample opportunity for these activities, such as laboratory classes, workshops and tutorials. In this cohort, the number of ways students connected did not impact on retention in their program. However, the quality and perceived value of these connections were not assessed, limiting interpretation of their impact on retention.

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Abstract: EF8

Embedding a clinical study in biomedical science teaching: Dual benefits for students and health research

Lisa Akison[1], Jacki Walker[1], Amelia Fotheringham[2], Gary Wilson[1], Scott Kershaw[1], Karen Moritz[1], Walter Thomas[1], and Linda Gallo[3]

[1]The University of Queensland, St Lucia QLD 4072, Australia
[2]Mater Research, South Brisbane QLD 4101, Australia
[3]The University of the Sunshine Coast, Petrie QLD 4502, Australia

There is strong evidence that learning is most effective when it occurs in circumstances resembling real-life, discipline-specific contexts (Herrington & Herrington, 2006). Authentic, inquiry-based activities enable students to take on roles and solve complex problems requiring critical thinking and decision-making (Zimbardi et al., 2015), with teachers and near-peer tutors helping to bridge the gap between theory and practice (Richards et al., 2020).

This case study describes a 3rd-year course in the Bachelor of Biomedical Science program where a cross-sectional clinical study has been embedded into practical classes since 2016. The study aims to obtain annual 'snapshots' of cardio-metabolic health in university students and identify potential risk factors for future chronic disease. Chronic health conditions such as obesity, type II diabetes and cardiovascular disease are major global and national concerns, with prevalence increasing worldwide. Social and behavioural risk factors, including poor diet and physical inactivity, contribute to this rise (AIHW, 2016) by underlying biomedical risk factors such as hypertension, obesity, dyslipidaemia and impaired glucose control. Estimating the prevalence of these risk factors early in adult life is critical to guide targeted health promotion and preventive strategies.

Ethics approval was obtained, and students provided consent for their class data to be included in the study. Data included direct measures of fasted cardio-metabolic markers in blood (e.g., glucose, insulin, HDL, LDL, triglycerides), anthropometric indices (including body composition via Bodpod), cardiovascular measures (blood pressure and heart rate), and self-reported diet and physical activity. To-date, we have collected data from >1,500 students contributing to three publications, with several more in preparation.

Students receive the de-identified dataset for their class and use this to test a hypothesis, chosen from six options. Therefore, in addition to contributing as study participants, they also take on the role of the researcher, making decisions on data analysis, handling spurious or missing data, and presentation of results. Their assessment is a report written in the style of a clinical research paper.

Responses from open-ended reflective questions at the end of the course indicate that students find this a valuable learning activity, both in deepening their understanding of how clinical research is conducted and in learning about their own metabolic health. By embedding a clinical study into teaching, this approach simultaneously enriches student learning and generates meaningful insights into the cardio-metabolic health of young adults.

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Abstract: EF9

Promotion of Physiology Education in East Africa by the EASPS

Nchafatso G Obonyo[1,2,3] and Abdullateef I Alagbonsi[1,4]

[1]East African Society of Physiological Sciences, East Africa, Rwanda. [2]Critical Care Research Group, The Prince Charles Hospital, Brisbane, Queensland, Australia. [3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia. [4]Department of Physiology, University of Rwanda, Kigali, Rwanda

Physiology education in Africa faces several challenges, including deficiencies in the contents of curricula used across many African Universities, a low number of physiology-training institutions, inadequate East African physiologists compared to their regional needs, *et cetera*. To address some of these problems, the East African Society of Physiological Sciences (EASPS) organized an inaugural conference aimed at transforming physiology teaching through competence-based curricula, launching the IUPS new physiology mentorship program in Africa, among other objectives[1]. For the first time, the EASPS developed the Physiology Curriculum Guideline for African Universities (PhysioCAFUN) as a first step to address these problems[2]. A committee of 15 Physiologists from East Africa (Rwanda, Tanzania), Southern Africa (South Africa, Zambia, Namibia), North Africa (Egypt, Sudan), West Africa (Nigeria, Senegal), Germany, and the USA was constituted to draft the PhysioCAFUN, and the draft was presented during a conference held in Tanzania in November 2023. Inputs from the conference were considered, and the approved version was published on the AAPS website.[2]

The PhysioCAFUN has 23 modules. Modules 1-15 cover organ systems, including principles and concepts of Physiology, molecular biology and cell physiology, cell-cell communication and control systems, integrated control of fluid-electrolyte and acid-base homeostasis, cellular neurophysiology, systems neurophysiology, and the physiology of endocrine, muscle, blood, immune, cardiovascular, respiratory, renal, reproductive, gastrointestinal and metabolic systems. Modules 16-23 cover optional contents, including environmental physiology, pharmacology, principles of animal experimentation and biomedical instrumentation, epidemiology, biostatistics and research methodology, clinical placement in laboratories, seminars on current topics in physiology, physiology entrepreneurship, and research project.

In conclusion, PhysioCAFUN serves as a freely available resource document for African stakeholders regarding the desired undergraduate physiology training and competencies. It will help universities in some African nations to freshly draft a curriculum suitable for their local needs, where there is a dearth of physiologists, or to benchmark and revise their curricula where physiology programs are already in place. We are collaborating with physiological societies across Africa to work with universities and stakeholders in their territories and persuade them of the gradual implementation of PhysioCAFUN. The EASPS has established a regional East African Physiology Quiz (EAPQ) that will assess the competencies of medical and dental students in the region using the learning outcomes prescribed in PhysioCAFUN. Other educational activities like the mentoring program, educational symposia and workshops, among others, will complement PhysioCAFUN to strengthen physiology in the region.

References:

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Abstract: EF10

Educator and Student Experience of Using Minecraft Edition in Physiology Education

Ben Perry, Gabriel Perrone, Chris Jones, Cherylea Browne, Kayte Jenkin

School of Science, Western Sydney University, Sydney, NSW

In tertiary education, the use of digital educational games has begun to increase in popularity in the past decade. Minecraft is a three-dimensional, open world “sandpit” game, where players have no set objective, but rather, are free to do as they wish within the physics of the environment, akin to “virtual Lego”. One fascinating quality of Minecraft is the ability to create functional circuitry and switches, which lends itself to teach physiological processes from a cellular to an organ level. Minecraft Education Edition was used within an on-campus collaborative educational activity in two separate second-year subjects covering skeletal muscle, cardiovascular, and renal physiology. This study will present the educator experience in creating and implementing Minecraft Education Edition, and data on the student experience via a HREC approved Qualtrics survey containing both Likert scale and open-ended questions (n = 26). Preliminary data indicates that 20 out of 26 (76.9%) of surveyed students either agreed or strongly agreed that Minecraft education Edition helped them reinforce content from the lecture, and only 3 out of the 26 (11.3%) agreed with a question stating that Minecraft Education Edition was a “waste of their time”. Overall, a strong majority of students reported Minecraft Education Edition provided perceived benefit to their study and retention of physiological processes. However, some students did not perceive any educational benefit whatsoever, indicating caution should be applied on overreliance on such an approach or not offering sufficient educational alternatives.

Abstract: ES1

Facing the fear: a scaffolding approach to teaching life sciences undergraduates R coding

Nadia Cerminara

School of Physiology, Pharmacology & Neuroscience, University of Bristol, UK

Big Data is prevalent in the biosciences with the advent of modern technology, resulting in large complex datasets. The ability to comprehend, manipulate, and visualize these datasets requires new computational tools, and increasingly necessitates researchers to have coding proficiency and the capacity to handle and scrutinize large datasets. In addition, alternative career paths often list coding as a desirable skill for applicants. We therefore need to equip students with the ability to work with large datasets through coding. Many of our physiology and neuroscience undergraduate students resist learning to code based on preconceived ideas such as having to be good at math. To overcome this, we propose a scaffolding approach using RStudio for data analysis, that has been implemented to teach basic coding skills to students in their first and second years of Neuroscience and Physiology programmes at University of Bristol (UK). We believe that this approach has been successful in lowering students' anxiety around coding and easing them into more complex tasks.

Abstract: ES2

From Synapse to Synopsis: The Tension Between AI-Assisted Learning and Critical Thinking

Angelina Y. Fong[1], Jennifer Lesi Zhu[1], Saw Hoon Lim[2]

[1] Depts of Anatomy and Physiology, [2] Biochemistry and Pharmacology, School of Biomedical Sciences, Faculty of Medicine, Dentistry, and Health Sciences, University of Melbourne.

The release of ChatGPT in November, 2022, has caused a seismic shift in discussion of higher education scholarship. The discourse in the literature has expressed concerns on the implications these Generative Artificial Intelligence (GenAI) technologies on critical thinking. There are significant concerns that introduction GenAI tools will erode critical thinking skills (Abouammoh et al., 2023; Ali et al., 2024). Research also suggest that GenAI tools can enhance student academic performance. Much of the research in the field has focused on student adoption of GenAI but have largely ignored the significant group of students who have intentionally avoided GenAI use in their studies. Furthermore, little is known about the biomedical science students' experience with these tools. Our current study utilised a mixed methods approach to examine biomedical science students' perceptions and experiences of GenAI.

We examined student declaration of GenAI use in two assignments (n=70). Most (>80%) students consistently reported GenAI usage in their assignment. No differences in grades were found between users and non-users (Assignment 1, $p = 0.41$; Assignment 2, $p = 0.46$). Next an online survey and focus groups were conducted with 2nd and 3rd year Physiology students. Of the 303 survey respondents, 249 (82%) students self-declared GenAI use, while 54 (18%) reported as Non-Users of GenAI. Non-Users reported greater certainty about institutional policy on GenAI, although both groups showed similar uncertainty on how GenAI assistance should be attributed within coursework, and broader concerns about its use. Thematic analysis of open-ended survey responses and focus group transcripts corroborated these patterns. Additionally, non-users cited distrust of GenAI's accuracy, fear of intellectual complacency, and ethical unease. In contrast, users reported efficiency in managing workloads, helpful learning support, psychological reassurance, and fear of being outcompeted by peers. Among users, verification appeared as a central strategy. Some found the process empowering and supportive, while others described it as burdensome and fatiguing. This results in a paradox of verification that we propose as a decision-making cycle that students must engage in during their studies that can create a positive outcome that foster critical thinking, but for some students can become overwhelming that result in cutting corners.

Ultimately, this study advances understanding of GenAI literacy by identifying the drivers and barriers of adoption in biomedical science. Moreover, it highlights that engaging with GenAI requires constant iteration. The findings expose an urgent need to embed GenAI literacy into biomedical education, providing evidence-based strategies for navigating an era defined by generative technologies.

Abstract: ES3

Competency hurdles for engagement and skill mastery in science & biomedical science education

Chantal Hoppe, Jack Mayhew, Yasith Mathangasinghe, Julia Young, Reyhan Akhtar, Kim Catania, Luca Fiorenza and Sonja McKeown

Department of Anatomy & Developmental Biology, Monash University, Clayton, VIC, Australia.

Rationale:

In an era of GenAI and evolving expectations for future workforce readiness, developing student confidence in hands-on, real-world skills has never been more critical, especially for non-vocational degrees. Across three large-scale undergraduate units exceeding 780 in embryology, 890 in introductory biology, and 640 in anatomy, we have implemented a competency-based assessment model designed to scaffold essential laboratory and psychosocial skills in a low-stakes, supportive environment.

Methods:

We employed simple pass/fail rubrics aligned to course-specific competencies, encompassing scientific proficiencies (e.g., microscopy, pipetting, dissection), academic skills (e.g., literature searching) and professionalism (e.g., communication, professional conduct, and ethical conduct) within low-stakes learning environments. Competency evaluations were embedded into weekly practical classes, with a focus on iterative feedback, structured engagement, and skill progression. The goal is to equip students with research-aligned and/or job-relevant competencies, while enabling development of skills through scaffolded, programmatic assessment.

Results:

Our preliminary analysis explores:

● The association between number of competencies attended, competency scores and final unit grades across the three disciplines

● Comparative analysis of professionalism scores

● Evidence of skill retention and engagement across second- and third-year progression of a cohort of students

● In-semester trends in student performance across the professionalism and technical skill domains

● Qualitative feedback from students

Conclusion:

The framework demonstrates a scalable, student-centred approach to competency development that can be adapted across disciplines to foster engagement, embed real-world skills, and enrich both the student learning experience and teaching practice

Abstract: ES4

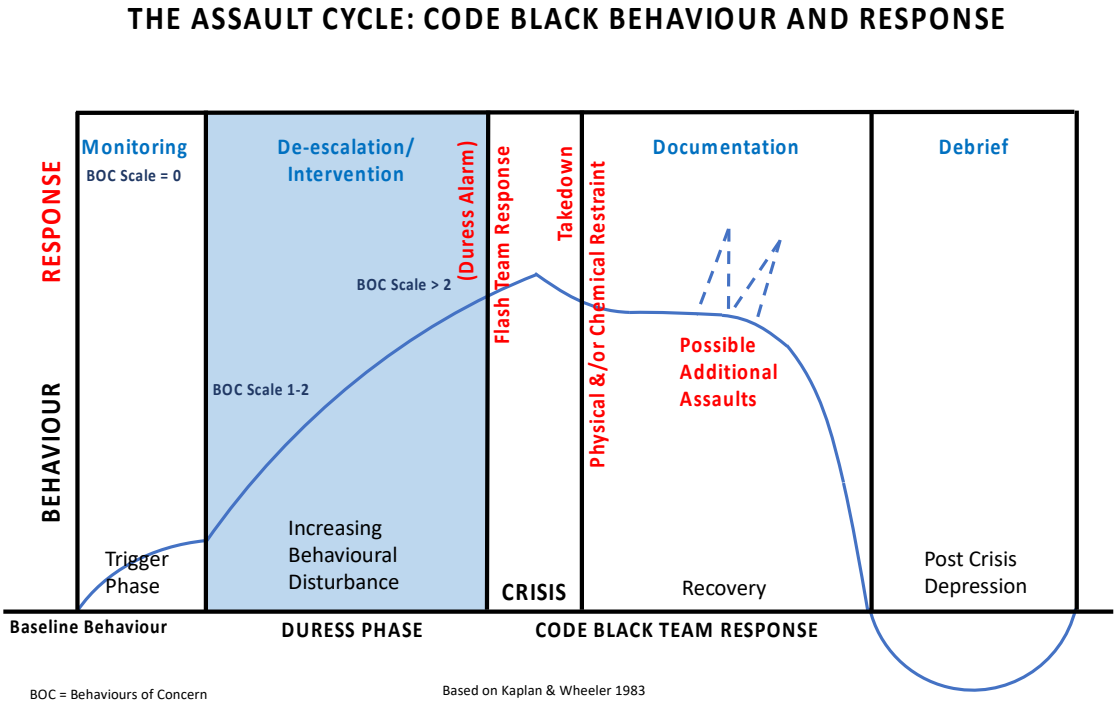
It's only tech. Designing assessment tasks with emerging tech at scale in med sci units

Martin Brown (Introduced by Andrew Moorhouse)

School of Medical Science, University of Sydney

Background

Students graduating as healthcare workers can feel unprepared for the demands of the clinical workplace. Training for many of the skills required in clinical practice is difficult to deliver at scale in a classroom environment. Traditional training methods consist of "on-the-job learning" and role-play. Emerging technologies enable educators to recreate convincing digital simulations of demanding clinical situations in a safe and supportive environment. This presentation describes the development of an effective training package in violence de-escalation for graduating and in-service clinicians using Virtual Reality (VR) and Artificial Intelligence (AI).



Methods

We used a constructivist methodology to evaluate the training needs of emergency department staff. We interviewed 20 clinicians and conducted a series of ethnographic observations in EDs across four hospitals in the Western Sydney Local Health District in New South Wales, Australia. We focussed on staff experiences of violent ("Code Black") events. Staff recounted experiences of 45 Code Black events which were collated and thematically analysed. From this analysis, we identified three training needs: exposure to a patient restraint, situational awareness and verbal de-escalation rehearsal. In response, we produced three interventions: a 360 video of a coordinated patient 'take-down', an interactive 360 video of an emergency department with patients exhibiting increasing levels of aggression, and an unscripted, AI-driven conversational agent for verbal de-escalation rehearsal. We tested these interventions on Nursing students.

Results

Post-intervention survey data indicated increased confidence in noticing behaviours of concern and applying verbal de-escalation skills.

Conclusions
Exposure to a live-action, 360 video in VR, within a supportive classroom setting, is a credible and scalable introduction to a patient restraint and effective in establishing situational awareness. Verbal interaction with a virtual agent is effective training in verbal de-escalation skills.

Abstract: ES5

Enhancing student engagement and performance through regular secure invigilated quizzes: A course assessment redesign

Luli Faber, Jemma Campbell, Laura Carniel and Walter G. Thomas

School of Biomedical Sciences, Faculty of Health, Medicine and Behavioural Studies, The University of Queensland, St Lucia, Queensland 4072, Australia.

Student engagement is an important predictor of academic success, improved learning outcomes, student retention, wellbeing and satisfaction, as well as success beyond higher education (Kahu et al, 2013). Assessment design plays a critical role in student engagement and learning outcomes. In the current academic environment, secure assessment practices that restrict the use of AI are essential to ensure that student performance accurately reflects individual capability.

In this study, we redesigned the assessment structure of a first year course, Physiology for Human Movement Students (PHYL1007), delivered in 2024, by replacing a mid-semester exam with regular, secure invigilated quizzes. Seven module-aligned quizzes were introduced, each worth 10% of the final grade, with the best five scores contributing to students' overall performance. This allowed flexibility, enabling students to miss or underperform in two quizzes without penalty. To support learning, practice quizzes were provided to reinforce key learning objectives. Secure assessment was ensured using Inspera in safe exam browser mode.

Student academic performance in 2024 was compared to the cohort in 2022 and 2023, prior to the assessment changes. At the end of semester, students were asked "How did you find the regular module workshops and quizzes in PHYL1007 this semester? How do you think they influenced your experience of PHYL1007 this semester, and how you'll approach your study going forward?" Engagement levels and drivers were assessed with the question, "On a scale of 1-10, how engaged did you feel with PHYL1007 this semester? Explain why you gave this score." Responses were subjected to inductive thematic analysis (Braun & Clarke, 2006).

A significant improvement in student performance was observed, with the failure rate decreasing from 23% in 2022 (n=252) and 20% in 2023 (n=315 students) to 7% in 2024 (n= 259). The average course grade increased from 64 ± 1% in 2022 (n=252) and 61 ± 1% in 2023 (n=315) to 70 ± 1% in 2024 (n=259, $p<0.001$). Students also achieved higher scores on the final exam (62 ±0.8%, n=308 in 2024 compared to 58 ±1%, n=245 in 2023, and 57 ± 1%, n=251 in 2022, $p<0.001$), despite it covering the entire course content in 2024 but only half of the course content in 2022 and 2023. Consenting students' responses (n=104) to open questions indicated that the new format helped students stay up to date with content (52% of students), enhanced learning (37%) and understanding of concepts (23%), and provided helpful feedback (31%), revision (30%) and exam preparation (23%). 85% of students reported moderately high or high engagement in the course, with the assessment changes being a key driver for self-reported engagement. Students also reported that the format influenced how they would learn in the future, encouraging regular study (40%), the use of practice quizzes (24%) and revision (13%).

Together, these findings show that frequent, meaningful assessments can enhance student engagement, improve learning outcomes, and reduce failure rates in undergraduate education. Conducting these assessments in a secure environment ensures that student performance is measured with accuracy and integrity.

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Abstract: ES7

Refreshing a Biomedical Curriculum

Scott Clarke and Julia Choate

Monash University, Melbourne Australia

The Bachelor of Biomedical Science at Monash University, Australia, was established in 1999 to educate and prepare students for careers in biomedical research. Core units in the degree program, comprising a third of the student's load, offer students a broad foundation in the biomedical sciences taught with a mix of lectures, workshops and laboratory classes; while elective units allow students to tailor their studies to their interests. These core units are taught by a large team of staff across multiple faculties, schools and departments. The course currently has a cohort of about 1800 students across the three years of the program. As a generalist degree not aligned to one specific profession, the graduate outcomes of our students are varied, with many students entering research, health professions, education and industry (Lee *et al*. 2025).

Over the past 25 years, there have been large changes in the biomedical science research and higher education sectors and expanding diversity of graduate outcomes (~60% of biomedical science graduates undertake further study in research or a health profession) .

To ensure the course remains contemporary, is future ready and is best suited to meet the needs of our students, throughout 2024 we developed a process to renew and refresh the Bachelor of Biomedical Science. The focus of the refresh is 1) development and sequencing of the 'core' curriculum with a focus on multidisciplinary, integrated approaches, 2) implementation of a course-wide program of assessment and 3) development of elective 'pathways' giving students greater agency in progressing towards their future careers.

A large educational change program such as this presented obstacles and tensions we needed to navigate. The course refresh process has been intentionally designed to incorporate multiple distinct stages and projects, processes for transparency and feedback, opportunities for key voices to be incorporated, and reporting of ongoing progress. Our refresh process may serve as a template for course-wide design and redesign projects in other contexts.

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Abstract: ES8

Verbal Vivas for Deeper Learning in Physiology

Alex M. Parker, Nilushi Karunaratne, Betty Exintaris.

Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia.

Introduction

With the rise of artificial intelligence (AI) technology, traditional assessments are particularly vulnerable to exploitation, which can lower the learning outcome to an information-gathering exercise and can undermine the level of conceptual understanding. Verbal viva is a formative in-person individual oral assessment where students respond to specific prompts given by the examiner in a question-and-answer format. The use of this assessment platform may circumvent AI vulnerability observed in traditional assessments and thus allows for a more authentic and accurate measurement of student learning. We sought to assess the efficacy of conceptual understanding in physiology via real-time reasoning-based communication in pharmacy students with the implementation of verbal viva.

Methods

First-year (n=338) undergraduate pharmacy students were given a choice to select a disease and medicine combination topic for research in preparation for the verbal viva. On the assessment day, students were asked to undergo a one-on-one interview-style format with an examiner, who asked questions based on their selected disease-medicine combination. To demonstrate a deep understanding of the chosen topic, students were asked to articulate how the diseased state deviates from normal function and how the treatment attenuates the diseased state using evidence-based reasoning.

Results

The verbal viva assessment format provided students with the opportunity to respond to interview-style questions in real-time in an evidence-based manner. Well-reasoned arguments were an effective indicator of students critically engaging with their preparation material and research, thus demonstrating a deeper understanding of their chosen topic. Of note, students were observed to exhibit increased stress and anxiety, given the direct face-to-face nature of the verbal viva assessment format. In addition, the timely release of student marks and the marking workload were significantly decreased as students were examined in real time.

Conclusion

Our observations suggest that the implementation of the verbal viva promotes pharmacy students to critically engage with the topic material for a more authentic and deeper learning in physiology. This is especially important in the rise of AI, ushering in opportunities to exploit vulnerabilities in traditional style assessments. The verbal viva platform can circumvent these vulnerabilities as the output requires real-time reasoning-based arguments, allowing for a more authentic evaluation of student learning. As a result, students are more engaged in the material for deeper learning in physiology education.

Abstract: FC1

Unravelling the role of the deubiquitinase Ubiquitin-Specific-Protease-15 (USP15) in skeletal muscle

Wayne X Du, Chris Karagiannis, Alastair AE Saunders, Scott T Carter, Hongwei Qian, Craig A Goodman and Paul Gregorevic

Centre for Muscle Research (CMR), Department of Anatomy and Physiology, The University of Melbourne, Victoria, Australia.

Post-translational modifications (PTMs) play a crucial role in regulating protein function, interaction, localization, synthesis, and degradation in skeletal muscle. One such modification is ubiquitination, which involves the attachment of a small protein, ubiquitin, onto a target protein via an E2-conjugating enzyme and an E3-ligase complex. Conversely, deubiquitinases (DUBs) are enzymes that remove ubiquitin from target proteins. While approximately 100 DUBs are encoded in the human genome, their roles in skeletal muscle remain poorly understood. In non-muscle tissues, the DUB Ubiquitin-Specific Protease 15 (USP15) has been shown to regulate components of the TGFβ signaling pathway, a key modulator of skeletal muscle physiology. This study aimed to investigate the role of USP15 in skeletal muscle under basal conditions and during TGFβ pathway inhibition by follistatin (Fst).

To assess the function of USP15 in skeletal muscle, we used recombinant adeno-associated viral vectors (rAAVs) to overexpress or knockdown USP15 (via shRNA) in the tibialis anterior (TA) muscle of healthy 7-week-old male C57Bl/6J mice (while anesthetized under 2-4% isoflurane). Overexpression and knockdown of USP15 were performed under basal conditions as well as in muscles undergoing Fst-induced hypertrophy. Additionally, proteomics and ubiquitinomics were performed on USP15 overexpression and knockdown muscles to identify potential targets of USP15. All tissues were collected from mice terminally anesthetized under 2-4% isoflurane.

Our results revealed that neither USP15 overexpression nor knockdown affected basal muscle mass or protein synthesis rates. However, they did influence the levels of Lysine-48 (K48)-linked ubiquitinated proteins, with USP15 overexpression decreasing and knockdown increasing K48 ubiquitination. Interestingly, proteomic analysis identified MuRF1, a well-known atrogene, as the most abundantly upregulated protein in USP15-overexpressing muscle, despite no observable change in muscle mass. Moreover, co-expression of USP15 with Fst significantly enhanced Fst-induced muscle growth, resulting in a 70% increase in muscle mass compared to 40% with Fst alone. Unexpectedly, MuRF1 expression was even higher in the USP15 + Fst group than in the USP15-alone group (~10-fold vs. 6-fold increase, respectively).

Overall, these findings suggest that USP15 regulates MuRF1 abundance and K48-linked ubiquitination in healthy skeletal muscle. Additionally, USP15 overexpression potentiates Fst-induced hypertrophy, offering new insights into the role of USP15 in muscle homeostasis and growth.

Abstract: FC2

Investigations into the Regulation and Role of Methyltransferase 21C in Skeletal Muscle

Scott Carter, Wayne X Du, Alastair Saunders, Chris Karagiannis, Hongwei Qian, Craig A Goodman, Paul Gregorevic

Centre for Muscle Research (CMR), Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia.

Introduction: Methyltransferase 21C (Mettl21C) is a lysine methyltransferase that is specifically enriched in skeletal muscle[1]. We recently identified Mettl21C as being upregulated in a proteomics screen of early (7d)-muscle growth induced by the TGF-β ligand inhibitor, Follistatin (FST), while Mettl21C mRNA was elevated by treatment with the anabolic selective androgen receptor modulator, GTX-024[2]. Conversely, we have shown that Activin A-induced muscle wasting was associated with decreased Mettl21C mRNA[3]. Mettl21C overexpression has been shown to increase muscle fibre size, which was associated with decreased protein breakdown[4], while knockdown decreased C2C12 myoblast differentiation and myotube diameter[5]. Overall, these data suggest that changes in Mettl21C may play a role in regulating muscle mass, however, more data from other models are required to confirm these hypotheses. In this study, we aimed to investigate how Mettl21C expression/abundance changed in models of muscle wasting, and whether knockdown or overexpression of Mettl21C would affect basal muscle mass.

Method: Mettl21C mRNA and protein were also investigated in previously collected muscle samples, including subcutaneous pancreatic cancer, Colon-C26 (C26) subcutaneous and 4T1 orthotopic breast cancer models of cancer cachexia, as well as unilateral peroneal nerve denervation. Eight-week-old C57B1/6J mice were anaesthetised with 4% isoflurane and maintained at 2% isoflurane for all surgical procedures, while meloxicam (2mg/kg) was administered subcutaneously as an analgesic. Mettl21C overexpression and knockdown were induced by the intramuscular injection of AAV6 viral vectors (5e9-1.2e11 vector genomes) into the tibialis anterior (TA) muscle. TA muscles were collected under anaesthesia 2- and 4-week post AAV injection. For inducible Activin-A cohorts, Mettl21C was overexpressed for 4 weeks, whilst Activin A expression (with Tet-Off tetracycline-responsive vector) was induced by removal of doxycycline chow after the first 2 weeks.

Results: Mettl21C was consistently downregulated at an mRNA level in all models of cancer cachexia, as well as at a protein level in the C26 model. Interestingly, Mettl21C mRNA decreased by 80% at a pre-cachectic timepoint (tumour palpation but no body mass loss) in male C26 mice and continued to reduce to endpoint, whereas female mice only had a significant decrease in Mettl21C mRNA at 25% body weight loss. Furthermore, Mettl21C expression was markedly lower in female control samples compared to male control samples. Interestingly, in the orthotopic 4T1 breast cancer cohorts, Mettl21C protein abundance was not decreased. Following denervation, Mettl21C abundance was significantly decreased after 7d and remained reduced at 14d. Neither overexpression nor knockdown of Mettl21C affected basal muscle mass at either of the 2- or 4- week timepoints. Finally, when Mettl21C was overexpressed prior to tetracycline mediated overexpression of Activin A, there was no impact on muscle mass compared to Activin A alone.

Conclusion: Collectively, our data identified that Mettl21C is consistently decreased across different models of muscle loss (denervation and models of cachexia). Furthermore, consistent with Mettl21C potentially being regulated by androgens, basal Mettl21C expression was higher in male vs female muscle. Mettl21C was not sufficient to regulate basal muscle mass, while Mettl21C overexpression had no impact on Activin-A mediated muscle atrophy, a condition in which Mettl21C expression is reduced. Further studies are required to examine whether Mettl21C is required for muscle growth.

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Abstract: FC3

The Contribution of Sex Hormones in Muscle Mass, Function, and Molecular Signalling in Females Aged 18-80

Annabel J. Critchlow[a], Danielle Hiam[a], Steven J. O'Bryan[b], Ross M. Williams[a], Megan Soria[c], Viktor Engman[a], Karel van Belleghem[a], Ross P. Wohlgemuth[d], Andrew Garnham[a], Christopher S. Fry[d], David Scott[a], Séverine Lamon[a]

[a]School of Exercise and Nutrition Sciences, Institute for Physical Activity and Nutrition (IPAN), Deakin University, Geelong, Australia.

[b]Institute for Health and Sport, Victoria University, Melbourne, Australia

[c]School of Life and Environmental Sciences, Deakin University, Geelong, Australia

[d]Department of Athletic Training and Clinical Nutrition, University of Kentucky, Lexington, Kentucky

Whether and how ovarian hormone fluctuations mediate the skeletal muscle response to ageing in females remains to be elucidated. We examined a tightly controlled, cross-sectional cohort of 96 females between 18-80 years of age to map the functional and molecular trajectory of muscle ageing and determine its relationship with female sex hormones. Across every decade, we quantified body composition using dual-energy x-ray absorptiometry, muscle morphology using peripheral quantitative computed tomography and voluntary and evoked muscle strength. Circulating sex hormone concentrations were measured with gas chromatography mass spectrometry and immunoassays. Morphology and gene expression of *vastus lateralis* muscle samples were assessed with immunohistochemical staining and RNA sequencing, respectively. After adjusting for the relevant variables, age was negatively associated with muscle mass, strength, and muscle fibre size, and positively associated with hybrid type I/II fibre prevalence and fibrosis. We found 37 unique patterns of gene expression across individual decades of age. Immune signalling, cellular adhesion, and extracellular matrix organisation pathways were the most upregulated with age, while mitochondrial function pathways were the most downregulated. Independently of age, circulating oestradiol and progesterone, but not testosterone, concentrations were positively associated with lean mass and negatively associated with hybrid muscle fibres across the lifespan. Oestrogen receptor binding sites were significantly enriched in upregulated genes in pre- versus post-menopausal muscle, suggesting a reduction in the translation of oestrogen target genes after menopause. The effects of sex hormone fluctuations across the female lifespan should therefore be considered in the development of therapies to mitigate age-related muscle wasting.

Abstract: FC4

Endogenous testosterone is associated with skeletal muscle fibre composition, but not muscle size or strength in males

Ross Williams, Annabel Critchlow, Briana Gatto, Viktor Engman, Karel Van Belleghem, Andrew Garnham, Danielle Hiam, Séverine Lamon

[1]Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Australia

Introduction: The relationship between sex steroid hormones and skeletal muscle mass and function remains ambiguous in males, with much of our knowledge stemming from research relying on exogenous manipulation of testosterone levels. This study aimed to address the association between endogenous sex steroid hormones and muscle outcomes across the male lifespan. A clearer understanding of this relationship will clarify testosterone's utility as a predictor for muscle loss in later life.

Methods: 82 biological males, aged 18-80 years and stratified by decade, participated in this study. Body composition was assessed via dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed topography (pQCT). Muscle strength was assessed using a leg press 5-repetition maximum (5RM) test. In a fasted state, a muscle biopsy was procured from the *vastus lateralis* and a venous blood sample taken. Testosterone and dihydrotestosterone were profiled using gas chromatography-mass spectrometry, and sex hormone-binding globulin measured via immunoassay. Free testosterone was estimated using the Vermeulen equation. Muscle samples were immunohistochemically stained to assess fibre morphology and type. All analyses were performed using RStudio (version 4.3.1).

Results: Appendicular lean mass index (ALMI) and muscle strength significantly decreased with age (β = -0.022; β = -2.467, both $p < 0.001$). Total testosterone and DHT did not significantly decrease with age and were not associated with any measures of muscle size or strength. Free testosterone decreased with age and was associated with measures of muscle size and strength; however, these relationships became non-significant following adjustment for age, dietary protein and training status. Total testosterone (β = −0.031 for type I; β = 0.031 for type IIa, both $p < 0.05$), free testosterone (β = −0.003 for type I; β = 0.003 for type IIa, both $p < 0.001$), and DHT (β = −0.306 for type I; β = 0.293 for type IIa, both $p < 0.05$) were significantly associated with fibre type proportions, independent of age, dietary protein intake, and training status. Additionally, free testosterone was significantly associated with type I fibre cross-sectional area (β = 0.001, $p = 0.015$) and showed a trend toward association with type IIa fibre cross-sectional area (β = 0.001, $p = 0.058$), following adjustment for the same covariates.

Conclusion: Our results suggest that while age remains the primary determinant of skeletal muscle size and strength, testosterone concentrations were significantly associated with muscle fibre type and size, independent of age and other covariates. Ongoing transcriptomic analysis aims to map muscle signalling pathways throughout the ageing continuum and determine the influence, or lack thereof, of sex steroid hormones on skeletal muscle gene expression across the male lifespan.

Abstract: FC5

Unravelling how biological sex and sex hormones shape the human muscle transcriptome across the lifespan

Karel Van Belleghem[1], Ross Williams[1], Annabel Critchlow[1], Viktor Engman[1], Megan Soria[2], Andrew Garnham[1], Janelle Browne[3], Danielle Hiam[1], Séverine Lamon[1]

[1]School of Exercise and Nutrition Sciences, Institute for Physical Activity and Nutrition (IPAN), Deakin University, Geelong, Australia.
[2]School of Life and Environmental Sciences, Deakin University, Geelong, Australia
[3]Hudson Institute of Medical Research, Clayton, Australia.

Introduction: Sex differences in skeletal muscle ageing arise from complex interactions between genetics, hormones, and environmental factors. Although the molecular and structural changes in muscle associated with ageing are becoming increasingly clear, the extent to which circulating sex hormones contribute to these differences remains unclear. By integrating transcriptomic and hormonal analysis, we aim to determine the contribution of sex hormones to sex-specific changes in ageing muscle.

Methods: A total of 178 adults (96 females, 82 males), stratified by decade of age, underwent comprehensive assessments including circulating sex hormone profiling and vastus lateralis muscle biopsies for immunohistochemical analysis and RNA sequencing.

Results: Principal component analysis revealed that sex was a primary driver of variance in the muscle transcriptome, while age contributed a more gradual, linear influence. Across the lifespan, 20 genes exhibited consistent sex-biased expression: most were Y chromosome-linked and expressed only in males, one (XIST) was female-specific, and three (TSPAN5, GRTP1-AS1, IRX3) were autosomal. These molecular differences were mirrored by structural changes in muscle: with age, males showed an increase in the proportion of Type 1 fibres and a decrease in Type 2a fibres, whereas females showed no significant changes. Additionally, fibre cross-sectional area declined with age for Type 1 fibres in both sexes and for Type 2a fibres in males, demonstrating that sex differences are evident not only in the muscle transcriptome but also in muscle architecture. Beyond sex influences, changes in age-related gene expression also affected pathways involved in immune regulation and protein turnover, with patterns differing between sexes.

Conclusion: These findings underscore the central role of sex chromosome–linked transcripts in establishing lifelong differences in skeletal muscle between males and females. Ongoing analyses will assess the extent to which circulating sex hormones influence muscle transcriptomic changes independently of sex, and how these molecular signatures relate to age-associated structural changes in muscle. By linking sex, hormones, and muscle biology, this study provides new insight into the mechanisms driving muscle ageing and their implications for health, disease risk, and physical function across the lifespan.

Abstract: FC6

Age related skeletal muscle and metabolic changes in response to a 3.5 day fast

Eloise K Tarry[1,2], Sarah S Mikkelsen[1], Melissa D'Ambrosio[3], Cathrine Tranberg[1], Emma B Fals[1], Flemming Dela[1,4], Christopher S Shaw[2], Steen Larsen[1,5,6] and Jørn W Helge[1]

[1]University of Copenhagen, Denmark. [2]Deakin University, Australia. [3]University of Pisa, Italy. [4]Riga Stradiņš University, Latvia. [5]Medical University of Bialystok, Poland. 6Copenhagen University Hospital – Bispebjerg and Frederiksberg, Denmark

Introduction: Peak fat oxidation (PFO) rates are determined by a combination of physiological factors with substrate availability, aerobic capacity, muscle oxidative capacity, age and sex playing major roles. Fasting is an effective method to induce short-term ketosis and manipulate rates of fat oxidation. Thus far, it is not known whether the age-associated decline in rates of fat use[1,2] persist in response to periods of acute ketosis. This study investigated alterations in substrate availability and the subsequent alterations in fat and carbohydrate oxidation during exercise following a 3.5-day period of fasting. It was hypothesised that peak fat oxidation rates would be substantially increased in both young and older healthy individuals in response to the fasting period, with an attenuated increase in older individuals.

Methods: Twenty-two moderately trained men were separated into a young *(n = 12)* (26 ± 4 yrs, BMI 24.1 ± 1.9, VO_{2peak} 53.6 ± 4.8 ml/kg/min) and older *(n = 10)* (63 ± 2 yrs, BMI 24.2 ± 1.2, VO_{2peak} 42.9 ± 6.5 ml/kg/min) (mean ± SD) group. Participants completed a 3.5-day water-only fast. Basal blood samples and a muscle biopsy from the vastus lateralis were collected before and after the fasting period alongside incremental exercise tests to determine PFO, maximal oxygen uptake and rates of carbohydrate and fat use. Two-way, repeated measures-ANOVA were used to assess differences between groups over time.

Results: Whole-body PFO increased from (0.46 ± 0.09 to 0.87 ± 0.15 g/min) and (0.42 ± 0.12 to 0.73 ± 0.16 g/min) in the young and older groups (main effect; time: $p<0.01$). Fasting-induced weight loss was similar in the young (84 ± 9 to 81 ± 9 kg) and older groups (76 ± 6 to 73 ± 6 kg) (main effects; time: $p<0.01$, age: $p=0.034$). Ketone levels were no different between age groups at baseline but were increased by fasting (time by age interaction: $p<0.001$). Ketone levels were lower in the young (2.4 ± 0.92 mmol/L) compared to older (4.7 ± 1.0 mmol/L) after fasting (post-hoc: $p<0.001$). Plasma FFA substantially increased in the young (218 ± 105 to 1289 ± 486 µmol/L) and older groups (357 ± 100 to 1634 ± 263 µmol/L) (main effect; time: $p<0.01$). Older individuals consistently displayed higher plasma FFA compared to the younger individuals, regardless of fasting (main effect; age: $p=0.018$). Skeletal muscle glycogen decreased by ~45% in both young (515 ± 126 to 286 ± 67 nmol/mg) and older groups (570 ± 215 to 294 ± 111 nmol/mg) (main effect; time: $p<0.01$). IMTG increased after fasting in the young (64.5 ± 24.9 to 81.2 ± 22.9 mmol/kg), and older individuals (90.4 ± 52.4 to 124.8 ± 64.5 mmol/kg) (main effects; time: $p=0.01$, age: $p=0.04$).

Conclusion: Peak fat oxidation rates were enhanced by approximately 2-fold following prolonged fasting which was accompanied by substantial shifts in substrate availability in plasma and skeletal muscle. The capacity to upregulate fat use during exercise in response to acute ketosis is preserved in older individuals.

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Abstract: FC7

Role of exercise-derived extracellular vesicles in regulating mitochondrial responses in skeletal muscle

Paula M Miotto, Song Chen, Jacqueline Bayliss, Benjamin L Parker, and Matthew J Watt

Department of Anatomy and Physiology, The University of Melbourne, Australia.

Exercise training leads to 'healthy' adaptations that are often associated with mitochondrial biogenesis and improvements in glucose and lipid metabolism. While there are well-established signalling networks involved in regulating exercise metabolism acutely, and adaptive responses to exercise chronically, it has been widely proposed that extracellular vesicles (EVs) secreted during exercise can promote adaptations to training. However, direct evidence to support the potential functional roles of exercise EVs remains limited. The purpose of this study was to examine the role of exercise EVs in regulating skeletal muscle mitochondrial function, cell signalling, and adaptive responses to exercise.

To determine the ability for exercise EVs to drive signalling events consistent with *acute* exercise, mice were administered a single i.p injection of EVs (30 µg) isolated (post-mortem) from blood of sedentary mice or mice that were acutely exercised (20 m/min, 10% grade, 1 hr). Control mice received saline or were acutely exercised. Experiments were approved by the Animal Ethics Committee at the University of Melbourne. Mice were deeply anesthetised with sodium pentobarbitol (50-150 mg/kg, i.p) and then killed by cervical dislocation prior to tissue collection and *ex vivo* analyses.

We found that acute treatment (1 hr post) with exercise EVs resulted in similar reductions in skeletal muscle mitochondrial ADP sensitivity (permeabilised muscle fibres, Oroboros) as mice that were exercised. Moreover, both exercise EV treatment and acute exercise reduced mitochondrial sensitivity to malonyl-CoA (M-CoA), a key inhibitor of carnitine palmitoyl transferase-1 (CPT-1), suggesting exercise EV treatment could lead to greater mitochondrial fatty acid transport. This was supported by greater skeletal muscle 14C fatty acid oxidation in exercise EV and exercised groups. PGC1α gene expression increased in response to exercise EVs or exercise, indicating a potential role of exercise EVs in signalling mitochondrial biogenesis. Interestingly, this occurred independent of known regulators of mitochondrial biogenesis during exercise, as exercise EVs did not promote mitochondrial reactive oxygen species production or activate traditional exercise signalling pathways (e.g. AMPK phosphorylation) in a similar manner to exercise.

To determine the importance of exercise EVs for facilitating mitochondrial adaptations in skeletal muscle with *chronic* exercise training, mice were treated i.p. with saline or an inhibitor of EV biogenesis (GW4869, ~2.5µg/g) 3-4 times per week for 4 weeks. During this time, mice remained sedentary or were exercise trained. While GW4869 treatment reduced circulating EV levels (nanoparticle tracking analysis), this did not prevent exercise-mediated changes in skeletal muscle mitochondrial function or impair mitochondrial biogenesis with exercise training (e.g., OXPHOS proteins).

Collectively, these data suggest that exercise EVs may acutely regulate mitochondrial responses in a similar manner to exercise, although reducing circulating EVs may not prevent skeletal muscle mitochondrial adaptions to exercise training.

Abstract: FC8

$GABA_B$ receptor regulation of membrane excitability and modulation of ion channels in human pluripotent stem cell-derived sensory neurons (hPSCs)

Arsalan Yousuf[1], Mitchell StClair Glover[2], Mirella Dottori[2], David Adams[2]

[1]Sydney Pharmacy School, University of Sydney
[2]School of Medicine and Health, University of Wollongong

$GABA_B$ receptor ($GABA_BR$) activation is involved in pain relief via reducing neuronal excitability in isolated rodent dorsal root ganglion (DRG) neurons (Bony *et al.*, 2022). Emerging strategies to develop, study and characterise human pluripotent stem cell (hPSC)-derived sensory neurons present a favourable alternative. In this study, hPSCs were differentiated into DRG sensory neurons using a chemical and transcription factor-driven approach. Molecular characterisation by immunocytochemistry and qPCR showed differentiated sensory neurons express key markers, such as BRN3A, ISLET1, and PRPH, in addition to $GABA_BR$ and ion channels, including $Ca_V2.2$, GIRK, and hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels. Functional characterization of $GABA_BR$ was carried out using whole-cell dialyzed and perforated patch clamp electrophysiology, with neuronal excitability studied under current clamp conditions in the absence and presence of $GABA_BR$ agonists, baclofen and α-conotoxin Vc1.1. Both baclofen and Vc1.1 significantly reduced membrane excitability by hyperpolarizing the resting membrane potential by ~-4 mV from -54 ± 4 mV (n = 10). In voltage-clamp mode, Vc1.1 and baclofen inhibited HVA Ca^{2+} channel currents by ~25% and 50% (n = 8), respectively, which was attenuated by the $GABA_BR$ antagonist CGP 55845. Hyperpolarization-activated current was inhibited by selective HCN antagonist ZD7288. Transient expression of human $GABA_B$ receptor subunits together with HCN1/2 channels in HEK293T cells confirm that baclofen and Vc1.1 potentiate HCN-mediated K^+ currents. Overall, this study reports $GABA_BR$ modulation of HVA Ca^{2+} channels and membrane excitability in hPSC-derived sensory neurons by baclofen and Vc1.1, which could pave the way for future cell models to study analgesic compounds.

Abstract: FC9

Paradoxical role of TRPC1/4 channels in the regulation of uterine contractility during pregnancy and in labour

Karen QW Leow[1], Olesia F Moroz[2, 3], Mary A Tonta[1], Marianne Tare[1,4], Harold A Coleman[1], Helena C Parkington[1]

[1] Department of Physiology, Monash University, Clayton, Victoria, Australia
[2] Department of Biotechnology, The State University, Kyiv, Ukraine
[3] Department of Biophysics and Neurobiology, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
[4] Monash Rural Health, Monash University, Churchill, Victoria, Australia

Background: Coordinated uterine contractions are critical for effective labour, ensuring safe delivery. Failure to progress in labour carries significant lifelong effects for both mother and infant. Despite its importance in pregnancy and birth, the myometrium has received comparatively less attention than other smooth muscles, delaying progress in identifying the detailed mechanisms that regulate myometrial contractility. This gap in knowledge limits our ability to properly manipulate contractions therapeutically during pregnancy and labour. Importantly, a better understanding of mechanisms underpinning the transition of the uterus from quiescence to a pro-contractile phenotype (within a day), critical during labour for successful delivery, is long overdue. The transient receptor potential canonical (TRPC) ion channels conduct sodium and calcium, thereby inducing depolarisation and contraction, and have been recognised as major regulators of smooth muscle activity across multiple organs. However, their role in uterine contractility remains understudied. An inhibitor and an activator of TRPC1/4 channels that are highly selective have recently been discovered. Our study aimed to investigate the role of TRPC1/4 channels in uterine contractility in pregnancy in rats.

Methods: Pregnant female Sprague Dawley rats were anaesthetised with isoflurane (5% in oxygen, delivered via an inhalation chamber) and humanely killed by decapitation on gestational day 18/19 (not in labour, NIL) and day 21 (in labour, IL). The uterus was removed, and myometrial strips of longitudinal smooth muscle were prepared. Membrane potential, using intracellular microelectrodes, was recorded simultaneously with contractility. Western blotting and immunohistochemistry were used to determine protein levels and identify the cellular location of proteins of interest. TRPC1/4 channel activity was manipulated using the selective antagonist, Pico145 (10 pM) (Rubaiy HN et al., 2017) and the selective activator Englerin-A (EA) (0.1nM-30nM) (Akbulut Y et al., 2015). All animal procedures were approved by the Monash University Animal Ethics Committee (AEC), in accordance with the National Health and Medical Research Council (NHMRC) of Australia guidelines for the use of animals in research.

Results: 1. Blocking TRPC1/4 channels using Pico145 significantly enhanced spontaneous contraction before and during labour. **2.** Pico145 also induced significant membrane depolarisation. This contradicts our expectation for blockade of a cationic channel. We considered that this result could be explained if Ca^{2+} influx through TRPC1/4 activates small-conductance Ca^{2+}-activated potassium channels (SK_{Ca}) expressed in the myometrium. The SK_{Ca} blocker, apamin (200 nM), induced depolarisation to the same extent as Pico145. In the presence of apamin, Pico145 induced a transient hyperpolarisation, supporting our expectation for cationic channel blockade. **3.** Low concentrations of the TRPC1/4 activator, EA, hyperpolarised the membrane, consistent with activation of SK_{Ca} channels. **3b.** At higher concentrations, EA induced depolarisation and contraction. These contractions were blocked by $CaCC_{inhib}$ (5μM), which inhibits the Ca^{2+}-activated chloride channel, ANO1. **4.** Pico145 depolarisation was markedly reduced IL. In contrast, TRPC1/4 activation by EA to induce depolarisation and contraction was more effective IL. These results are consistent with a reduction in the contribution of Ca^{2+} from TRPC1/4 to activate SK_{Ca} channels during labour.

Conclusion: Throughout pregnancy, TRPC1/4 channels play an important role in regulating uterine contractility. TRPC1/4-mediated Ca^{2+} influx activates SK_{Ca} channels, maintaining uterine quiescence during pregnancy. This mechanism is diminished during labour, permitting the strong uterine contractions necessary for successful delivery. Enhanced Ca^{2+} entry through TRPC1/4 channels upon activation at higher concentrations of EA likely activates ANO1 channels, which supports the depolarisation required for contraction IL. These channels may be a potential therapeutic target for promoting effective contractions during labour and managing labour-related complications.

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Abstract: FC10

Harnessing uterine potassium channels to improve labour outcomes for women

Helena C Parkington[1], Mary A Tonta[1], Shaun P Brennecke[2,3], Penelope M Sheehan[2], Harold A Coleman[1]

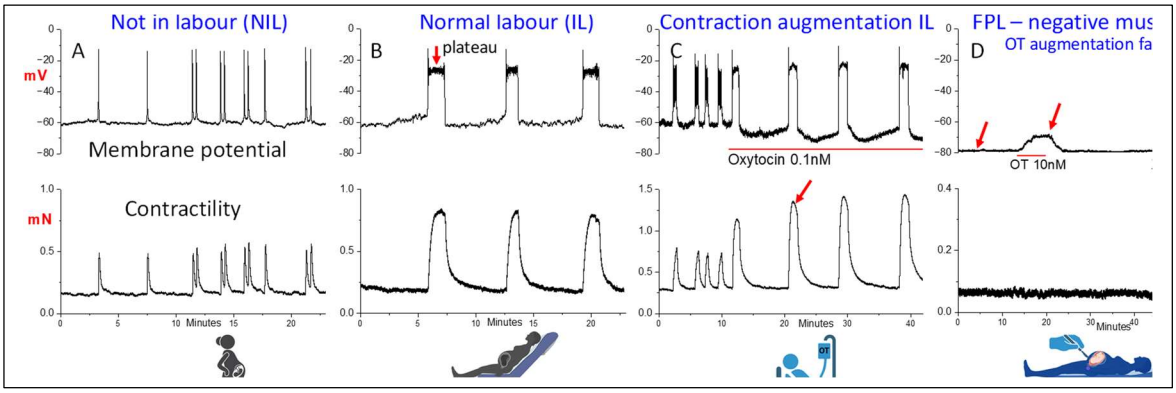
[1]Department of Physiology, Monash University, Clayton, Victoria, Australia. [2]Department of Perinatal Medicine Pregnancy Research Centre, Royal Women's Hospital, Parkville, Victoria. [3]Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia

Background: In 2022-23 39% of Australian pregnant women underwent caesarean delivery (CD), 60% of those for poor contractions (failure to progress in labour, FPL). For the mother: CD-associated short and longer-term risks include: a 7-fold increased chance of preterm birth in subsequent pregnancies, 3.4-fold increased risk of hysterectomy, 3-fold for postpartum haemorrhage and 2-fold for postpartum depression (10% increase over the past 10 years). For the neonate: there is a 3-fold increased risk of immune, metabolic and cardiovascular issues throughout childhood and adolescence.

Methods: We studied myometrial tissues from 3 groups of women: (i) term not in labour, (ii) normal labour, (iii) in labour but failing to progress (10 women/group). We immediately studied the contractile mechanisms in a tissue sample using intracellular electrophysiological and contractility recording. We subjected other samples to RNA sequencing and Western blotting. All women had body-mass index (BMI) $\leq$ 26 and were $\leq$ 36yrs. This study was approved by the Research Ethics Committee of the Royal Women's Hospital, adhering to the guidelines of the Declaration of Helsinki. Participants provided informed written consent for myometrial sample collection prior to surgery/tissue collection.

Results: As pregnancy advances, resting membrane potential becomes progressively less negative, resulting in progressive facilitation of action potential firing and contraction. At term, but not in labour (NIL) action potentials (AP) are brief, resulting in small brief contractions (Fig 1A). In normal labour (IL) the AP spike is followed by a prolonged plateau of depolarization resulting in the robust contractions required for labour (Fig 1B). In groups (i) and (ii) oxytocin (OT) augmented the plateau and improved contraction (Fig 1C), which would permit vaginal delivery as required. In group (iii) labouring women, tissue membrane potential was so negative that OT augmentation failed, necessitating CD in labour. A potassium (K) channel blocker (XE991) relieved the negativity, enabling APs to occur implicating a voltage-gated K channel (Fig 1D). We progressed the question of K channel identity by performing RNA-seq analysis of myometrium obtained during normal IL and in FPL. Excitingly, our RNA-seq data found stronger expression of 2-pore K channels previously unknown in human myometrium, and enhanced protein expression of Kv7.3 and Kv4 channels. There was an increase in mRNA for many K channels in FPL tissues (KCNK13, KCNK3 and KCNQ3). This explains the deep negativity in FPL. Membrane potential was very negative in some labouring women (Fig 1D), and these had CD because of FPL. Western blotting confirmed greater protein synthesis for these K channels.

Conclusions: 24% of Australian women FPL, necessitating CD. This is commonly due to (i) high BMI at conception or (ii) older age (>36 years) at first pregnancy. Oxytocin receptors are commonly low in women with high BMI, and we previously identified a regulation failure of the K_V11 hERG K channel which prevents the development of the AP plateau in these women (Parkington et al, 2014). We now identify a profound failure of the membrane potential depolarization required for APs to achieve threshold in the labouring myometrium of young, lean women and demonstrate that blockers of these channels can relieve the consequent negativity of the myometrial membrane. We are testing new potential drugs that might alleviate



this problem.

Reference: Parkington et al. (2014) *Nature Communications* 5:4108.

Abstract: FC11

An electrophysiological assay to study the diverse functional properties of pathogenic *CACNA1A* variants

Cameron Adly[1], Philip K Ahring[1], Han Chow Chua[2]

[1]School of Medical Sciences, Faculty of Medicine and Health, Brain and Mind Centre, The University of Sydney, Sydney 2006 New South Wales

[2]Sydney Pharmacy School, Faculty of Medicine and Health, Charles Perkins Centre, The University of Sydney, 2006 New South Wales

CACNA1A encodes the pore-forming subunit of the P/Q type calcium channel $Ca_V2.1$, a key player in neurotransmission. *CACNA1A* variants can lead to a wide range of debilitating disorders including episodic ataxia, familial hemiplegic migraine and developmental and epileptic encephalopathies. Despite over 2,000 suspected pathogenic variants reported to date, the functional consequences of most of these variants have yet to be determined, limiting precision medicine approaches. This challenge is exacerbated by poorly validated assays leading to conflicting variant interpretation, hindering coherent genotype-phenotype correlation of *CACNA1A* disorders. To address this gap, we aim to develop a medium-throughput two-electrode voltage clamp assay in *Xenopus laevis* oocytes (from anaesthetised frogs) to aid variant classification. We first tested the recurrent pathogenic variants R198Q (loss-of-function) and A712T (gain-of-function) and found clear differences in multiple parameters such as maximal current amplitudes, half-maximal potentials ($V_{1/2}$) for activation and inactivation, thus validating the dynamic range of our assay. To our surprise, a previously characterised loss-of-function variant G230V showed hyperpolarised $V_{1/2}$ activation and decreased inactivation in our assay, consistent with a gain-of-function variant profile. As the accurate assignment of pathogenicity for disease variants directly impacts treatment decisions, our findings highlight the need for a well-validated assay to assess variant pathogenicity accurately.

Abstract: FC12

Fractional anisotropy measured from Diffusion Tensor MRI identifies neuroprotection in a severe traumatic brain injury rat model with the first-in-class TRPC channel blocker Xolatryp™

Georg von Jonquieres[1], Jasneet Parmar[1,2], Andre Bongers1, Simone Zanoni[1], Brendon Lee[1], Jignesh D Pandya[3], Anke H Scultetus[3], Hiren R Modi[3], Sudeep Musyaju[3], Alexandra Suchowerska[1,2], Phillip Coghlan[2], Benjamin J Evison[2], James A Bonnar[2], Gary D Housley[1]

[1]Translational Neuroscience Facility and Department of Physiology, School of Biomedical Sciences, UNSW & Biological Research Imaging Laboratory, Mark Wainwright Analytical Centre, UNSW, Sydney, Australia; [2]Nyrada Inc, Sydney Australia, [3]Walter Reed Army Institute of Research (WRAIR), Brain Injury Neuroprotection Branch, Silver Spring, MD, USA.

Canonical transient receptor potential (TRPC) channels are broadly expressed in the CNS and are activated by Gαq - type metabotropic receptors, including the class I mGluR. These non-selective cation channels mediate a significant component of Ca^{2+} loading in neurons with sustained glutamate exposure, where reduction in this Ca^{2+} burden in a TRPC1,3,6,7, quadruple knockout mouse model of focal ischemic brain injury was protective (Parmar et al. Transl. Stroke Res. 2024). Xolatryp™, aTRPC channel blocker matched to this target has recently cleared phase I clinical safety studies and potential efficacy was evaluated in a rat penetrating TBI model established at WRAIR.

Under a WRAIR animal ethics approved protocol, Sprague-Dawley rats (males) were anaesthetised using isoflurane and subjected to a transient pneumatic expanding probe in the post-striatal region. Intravenous treatment with Xolatryp™ (5mg/kg/h) or vehicle control commenced within 30 minutes post-injury and was sustained for 48 hours through the acute – subacute phase of the penetrating TBI (n = 13 vehicle, n = 15 drug). Following euthanasia and transcardial perfusion with paraformaldehyde, the intact skulls were shipped to UNSW under refrigeration and the cranium immersed in a 0.02% (v/v) gadolinium-based contrast agent for a week. Brains were then imaged in the 9.4T small animal MRI (Bruker BioSpec Avance 94/20) using established coronal T2 weighted structural and diffusion tensor imaging (DTI; 30 gradient directions, b=1000s/mm^2) protocols. The fractional anisotropy (FA) was calculated from the DTI and analysis used mid-level transects, where the contralateral (uninjured) hemisphere in coronal slices served to normalize tissue integrity. In the Xolatryp™ - treated group our analysis identified an extended area of multiple consecutive 1 mm slicesin the posterior half of the brain injury track where the FA values were significantly higher in the injured hemisphere compared to the vehicle control cohort, indicative ofimproved brain tissue integrity. These findings support the efficacy of Xolatryp™ as a novel first-in-class TRPC channel blocker for brain injury neuroprotection.

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Targeted drug screening in a *Drosophila* model of *KCNT1* epilepsy

Rashid Hussain[1], Chiao Xin Lim[1], Zeeshan Shaukat[1], Grigori Y Rychkov[1,2,3], Michael G Ricos[1], and Leanne M Dibbens[1]

[1]Epilepsy Research Group, Clinical and Health Sciences, Australian Centre for Precision Health, University of South Australia, Adelaide, South Australia.
[2]School of Biomedicine, University of Adelaide, Adelaide, South Australia.
[3]South Australian Health and Medical Research Institute, Adelaide, South Australia.

KCNT1, a Na^+- activated K^+ channel (also known as SLACK, $K_{Ca}4.1$ or Slo2.2), is a major contributor to the Na^+-activated K^+ current ($I_{K_{Na}}$) which down regulates neuronal excitability. Following a rise in intracellular [Na^+], KCNT1 channels open to increase K^+ conductance and to prolong the slow afterhyperpolarisation following an action potential, thereby reducing the chance of repetitive neuronal firing [1]. Mutations in *KCNT1* have been identified in a range of epilepsies with drug-resistant seizures and are the major cause of epilepsy of infancy with migrating focal seizures (EIMFS), where cognitive and developmental regression follow seizure onset [1,2]. *KCNT1* mutations also cause other severe epilepsies beginning in infancy, including West Syndrome and Ohtahara Syndrome, as well as a range of focal epilepsies, including sleep-related hyper-motor epilepsy [1,3]. All epilepsy-causing *KCNT1* mutations are gain-of-function, significantly increasing K^+ conductance compared to WT KCNT1 [4]. There are currently no effective treatments for *KCNT1*-epilepsy, however, considering that abnormally large KCNT1 K^+ conductance is the underlying cause of the disease, specific KCNT1 blockers may provide a way of treating *KCNT1*-related seizures.

In this study we developed a *Drosophila* model of *KCNT1*-epilepsy and investigated if the attained seizure phenotype responds to some of the drugs currently used to treat patients with *KCNT1*-epilepsy. To generate the *Drosophila* models, human *KCNT1* with the patient mutation G288S, R398Q or R928C was introduced into *Drosophila* by transgenesis. The mutant KCNT1 channels were expressed in different neuronal types, including GABAergic neurons, and flies were investigated for a seizure phenotype using bang sensitive behavioural assays. *Drosophila* used for seizure analysis were grown on food containing between 0.001 µM and 1 µM of the following commonly used anti-epileptic drugs: cannabidiol (CBD), vigabatrin, valproic acid, carbamazepine or quinidine [5]. Flies grown on the same food with the addition of the vehicle (ethanol, 1µl/1ml) were used as a control. In parallel, these drugs, except quinidine, were investigated for their ability to block WT and mutant KCNT1 channels expressed in HEK293T cells using patch clamping.

Drosophila lines expressing mutant G288S, R398Q and R928C KCNT1 in GABAergic neurons each showed a statistically significant seizure phenotype, while expression of WT human KCNT1 did not [5]. R398Q gave the strongest seizure phenotype with 48% of animals showing seizure activity followed by G288S with 41% and R928C with 38%. Carbamazepine, valproic acid and quinidine, each exacerbated the seizure phenotype in the three KCNT1 mutant *Drosophila* lines, whereas vigabatrin had variable effects. Only CBD showed a significant reduction of the seizure phenotype in all three *KCNT1* mutant lines and showed a dose-dependent response. In patch clamping experiments, carbamazepine, valproic acid and vigabatrin had no effect on KCNT1. In contrast, CBD inhibited WT, R398Q, R928C, and G288S KCNT1 with IC_{50}s of 5.6 ±1.88 µM (n=4), 0.41± 0.22 µM (n=4), 0.47 ±0.31 µM (n=4), and 0.81± 0.34 µM (n=4), respectively.

In summary, our study shows that the expression of patient-specific *KCNT1* mutations in *Drosophila* give a seizure phenotype, modelling human *KCNT1*-epilepsy. The attained seizure phenotypes were affected by the addition of drugs currently used to treat people with *KCNT1* epilepsy, suggesting that the *Drosophila KCNT1* models may be useful as preclinical tools for screening new drugs.

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Abstract: FC14

Help or hinderance, are CryoEM structures sufficient evidence for precision medicine in epilepsy?

Anushka Goswami[1], Philip K Ahring[2], Sebastian Ortiz[3,4], Susan XN Lin[3], Rikke S Møller[3,5], Nathan L Absalom[1,2]

[1]School of Science, Western Sydney University. [2]Brain and Mind Centre, University of Sydney. [3]Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, member of the ERN-EpiCARE, Dianalund, Denmark. [4]Institute of Clinical Medicine, University of Copenhagen, Denmark. [5]Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

Epilepsy is a neurological disorder with diverse genetic and pharmacological mechanisms. As the number of identified genetic variants in pediatric epilepsy grows, opportunities have emerged to develop precision medicines that directly target the genetic cause. Recent cryo-EM structures of $GABA_A$ receptors from epileptic brain tissue revealed that levetiracetam and lamotrigine bind to an allosteric site, enhancing receptor activity similar to benzodiazepines (Zhou et al. 2025). This project aims to determine whether levetiracetam or lamotrigine can serve as effective precision therapies for epilepsy-associated $GABA_A$ receptor variants that impair receptor function or expression.

To address this, we examined clinical outcomes and side-effect profiles associated with lamotrigine, levetiracetam, sodium channel modulators, and GABA-pathway modulators, and compared them as indirect measures of drug mechanism. Clinical survey analysis revealed that benzodiazepines effectively reduced seizures at individuals with a $GABA_A$ receptor loss of function (LOF) variant, as expected of a drug directly increasing $GABA_A$ receptor activity, but were ineffective at gain of function (GOF) variants. Levetiracetam, but not lamotrigine or sodium channel inhibitors, was similarly effective at LOF but not GOF variants.

However, neither lamotrigine nor levetiracetam displayed the strong sedative profile observed with GABAergic modulators, with somnolence exclusively reported by benzodiazepines selective for the allosteric site at $GABA_A$ receptors. suggesting that their clinical activity does not reflect occupation of the same binding site. This clinical evidence indicates that the therapeutic actions of lamotrigine and levetiracetam remain consistent with their canonical mechanisms—sodium channel modulation and synaptic vesicle 2A binding—rather than $GABA_A$ receptor allosteric potentiation.

This data demonstrates that levetiracetam and lamotrigine are not suitable precision therapies for LOF $GABA_A$ receptor variants, at odds with the cryo-EM structural data. It is possible that the cryo-EM densities attributed to lamotrigine and levetiracetam reflect low-occupancy, non-specific, or artifactual binding, or even represent averaged densities from multiple compounds present in patient tissue samples. To determine whether direct $GABA_A$ receptor modulation underlies the mechanism of action of lamotrigine and levetiracetam, we are currently determining the potency and efficacy with two-electrode voltage clamp electrophysiology. Nevertheless, this study highlights the need for caution in extrapolating drug mechanisms directly from cryo-EM maps.

Reference: Zhou, J., Noviello, C.M., Teng, J. et al. Resolving native GABAA receptor structures from the human brain. Nature. 638, 562–568 (2025).

Different actions of RyR2 open and closed channel block explained by a multiscale Ca^{2+} release model

Morris Vysma[1] and Derek R Laver[2]

[1]School of Engineering, University of Newcastle, Callaghan, NSW, Australia. [2]School of Biomedical Sciences and Pharmacy, University of Newcastle and Hunter Medical Research Institute, Callaghan, NSW

The release of Ca^{2+} from the Sarcoplasmic Reticulum (SR) of cardiomyocytes is central to both cardiac muscle contraction in response to action potentials and to cardiac pace making. During systole, Ca^{2+} is released from the SR via clusters of Ca^{2+}-activated ryanodine receptor Ca^{2+} channels (Type 2 ryanodine receptors, RyR2) in the SR membrane of the dyad (synapse of the SR and sarcolemma membranes). This release of calcium strongly reinforces local RyR2 activation, a process called calcium-induced calcium release (CICR). Eventually, Ca^{2+} release is exhausted and during diastole, Ca^{2+} is sequestered back into the SR by the ATP powered Ca^{2+} pumps (SERCA2a) in the SR membrane [1]. The NCX, that extrudes 1 Ca^{2+} in exchange for 3 Na^{+}, contributes to depolarisation of the sarcolemma, thus providing a link by which Ca^{2+} release can advance or retard spontaneous action potentials.

Cardiac action potentials normally drive the cyclic release and uptake of Ca^{2+} by the SR in ventricular cells. However, pathological over-activation of RyR2 due to inherited mutations in RyR2, certain drugs or by chemical modifications of RyR2 following ischemia or during heart failure cause heart arrhythmias and sudden cardiac death [2]. However, it is unclear how changes in RyR2 activity can underlie sustained pro- or anti-arrhythmic affects against a background of “autoregulation” of SR Ca^{2+}. A decrease in RyR2 activity should cause a decreased Ca^{2+} leak that will load the SR until the Ca^{2+} leak once again balances the Ca^{2+} uptake through SERCA2a. In accord with this idea, tetracaine, a closed channel blocker that increases RyR2 closed durations, only causes a transient depression of SR Ca^{2+} leak, leading to a pro-arrhythmic action. However, flecainide, an open channel blocker that decreases RyR2 open durations, inhibits RyR2 activity without changing SR Ca^{2+} load and has a sustained anti-arrhythmic action [3]. Clearly, the role of RyR2 in arrhythmia cannot be understood against a simple framework of quasi steady-state Ca^{2+} fluxes and must incorporate RyR2 gating kinetics. We present a multiscale, dynamic model for Ca^{2+} sparks in permeabilised cardiomyocytes using experimentally determined RyR2 gating kinetics that explains the very different actions of open and closed channel block of RyR2 on SR Ca^{2+} release.

Ca^{2+} sparks are thought to be the basic quanta of global SR Ca^{2+} release. They are brief, localised Ca^{2+} release events at single dyads that were first observed with fluorescent confocal microscopy by Cheng et al., [4]. The model simulates 15,000 Ca^{2+} release sites in cytoplasmic arrays informed by super-resolution micrographs [5]. Details of computations of dyad Ca^{2+} release, Ca^{2+} diffusion, buffering have been published previously [6].

Our multisite model predicts the initial rise in spark fluorescence ($[Ca^{2+}]$) to result from a regenerative phase of CICR between RyR2 within a dyad. The decline and termination of spark fluorescence occurs as SR $[Ca^{2+}]$ near the release site (terminal SR) declines to ~10% of resting levels, which reduces the Ca^{2+} flux through RyR2s and hence dyad $[Ca^{2+}]$ to a point where RyR2s start to deactivate, further reducing the Ca^{2+} efflux in an induction-decay cycle that continues until Ca^{2+} release ceases all together. We find that both RyR2 open and closed blockers produce only small changes in spark morphology. However, in accord with experimental findings [3], only closed blockers caused a transient decrease in spark frequency and SR Ca^{2+} release that lasted until store load increased again by autoregulation. Thus, in steady state, RyR2 closed block increased SR $[Ca^{2+}]$ and spark amplitude whereas RyR2 open block had relatively little effect on SR $[Ca^{2+}]$.

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Molecular control of hepatic ureagenesis with dietary protein enrichment

Adam J Rose[1], Patricia M Rusu[1], Andrea Y Chan[1], Yuqin Wu[1], Maria Almira Cleofe[1] & Stefan Bröer[2]

[1]Nutrient Metabolism & Signalling Laboratory, Department of Biochemistry and Molecular Biology, Metabolism, Diabetes and Obesity Program, Biomedicine Discovery Institute, Monash University, Clayton, Australia.
[2]Research School of Biology, Australian National University, Canberra, Australia.

In terrestrial animals, matching excess nitrogen waste disposal by the metabolic process known as ureagenesis is critical for proper physiological function. The liver is the only organ that has the complete urea cycle and is the chief site of urea production for eventual excretion into the urine by the kidneys. It is well known that urea production is matched to dietary protein supply. Indeed, using a range of equicaloric diets varying in dietary protein:carbohydrate ratio, we show that mice exquisitely adapt their rate of ureagenesis to protein intake. However, the molecular physiological mechanisms involved in this metabolic coordination are not known. Using *ex vivo* experiments of precision cut liver slices from mice adapted to differing dietary protein amounts (tissue collected from dead animals humanely killed by cervical dislocation), we show that while amino acid supply and the peptide hormone glucagon can acutely increase ureagenesis, the major determinant is a chronic adaptation in the hepatic ureagenesis machinery, including that of a neutral amino acid transporter. Hepatocyte-selective silencing of this amino acid transporter blocks ureagenesis in response to a protein-enriched diet. In summary, here we demonstrate that chronic adaptations of the ureagenesis machinery play a dominant role in the adaptation to a protein-enriched diet, with amino acid transport being a rate limiting step.

The Secondary bile acid Taurodeoxycholic acid may predict, but paradoxically arrest the progression of, hepatocellular carcinoma

Benoit Smeuninx[1], Ahlam Hawsawi[1], Saskia Reibe[2], Yen C Koay[3], John F. O'Sullivan[3], Michael Karin[4] & Mark A. Febbraio[1]

[1]Cellular and Molecular Metabolism Laboratory, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia. [2]Medical Sciences Division, Oxford University, UK, [3]Heart Research Institute, Australia. [4]Department of Pharmacology, UCSD, USA.

Background: Hepatocellular carcinoma (HCC) is one of the most common and deadly cancers worldwide. Early diagnosis increases the likelihood of successful interventional surgeries such as tumour ablation or resection. The swift and asymptomatic progression of the disease, however, renders these treatment options ineffective. As such, discovery of *bona fide* disease biomarkers and the development new and effective treatments are clinically unmet needs.

Methods: Accordingly, we utilised the transgenic *MUP-uPA* mouse model of metabolic dysfunction-associated steatohepatitis (MASH) driven HCC to (1) explore and (2) validate new disease biomarkers. When fed a high-fat diet, all *MUP-uPA* mice develop MASH at 24 weeks of age (WOA), whilst only ~60% will develop HCC at 40 wk of age. This partial penetrance model renders it ideal for disease biomarker discovery and validation. *MUP-uPA* mice were fed an HFD from 6 WOA, blood samples obtained at 24 WOA, and mice humanely killed by cervical dislocation (under 5% isoflurane) at 40 WOA. Untargeted metabolomics were performed on the 24 WOA plasma samples and segregated into those that did or did not develop HCC at 40 WOA.

Results: Despite all mice being phenotypically identical at 24 WOA, plasma levels of the secondary bile acid, taurodeoxycholic acid (TDCA), were ~10-fold higher (*P<0.01*) only in those mice that ultimately developed HCC. These data were next confirmed in a cohort of human blood samples where TDCA levels were elevated in HCC patients with fibrosis compared with healthy individuals and patients with metabolic dysfunction-associated fatty liver disease (MAFLD) and cirrhosis, and HCC with fibrosis patients (P<0.01). To determine the effects of TDCA on disease progression, we next examined whether supplementation via the drinking water was able to increase circulatory TDCA levels. As such, circulatory TDCA levels were increased upon a 2-week supplementation period (4.8mg/kg/week) in male C57BL/6 mice. Next *MUP-uPA* mice were fed an HFD from 6 WOA and either supplemented with or without TDCA in the drinking water (6.4mg/kg/wk) from 7 WOA for the duration of the study. At 24 WOA, mice were anaesthetised using isoflurane (5%) and a blood and liver biopsy sample were obtained. *MUP-uPA* mice were aged a further 16 wk and humanely killed by cervical dislocation (under 5% isoflurane) at 40 WOA. At 40 WOA, TDCA supplementation decreased mRNA markers of liver inflammation (P<0.05 for all markers) and ER stress (P<0.05 for all markers), decreased (P<0.05) liver fibrosis and a trend towards a slower HCC disease progression as observed by a lower number of *MUP-uPA* mice presenting with advanced HCC.

Conclusion: Elevated levels of circulating TDCA in patients with MASH may be indicative of future HCC development. Paradoxically, when TDCA was supplemented to *MUP-uPA* mice fed an HFD, HCC disease progression was arrested. These data indicate that TDCA can both be utilised as a disease biomarker and a therapeutic target for treatment.

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The role of activin A in renal and skeletal muscle pathophysiology in kidney disease

Alexandra E Walker, Ben D Perry, Kayte A Jenkin

School of Science, Western Sydney University, Locked Bag 1797, Penrith NSW 2751.

Rationale: Chronic kidney disease (CKD) is a substantial global health concern, impacting 10-15% of the population. Renal fibrosis, and the structural and functional changes that accompany it, are a key step in the pathogenesis of CKD. Intriguingly, the effects of CKD are not limited to the renal system; skeletal muscle wasting is common in CKD and synergistically causes debilitation and reduced quality of life. We need to identify new therapeutic targets that effectively ameliorate CKD development and progression, such as those that target renal fibrosis and muscle wasting. Upregulated Activin A, a growth and differentiation factor, has been identified in CKD pathologies. Whilst inhibiting Activin A successfully ameliorates renal fibrosis and muscle wasting *in vivo*, understanding of its signalling pathways remains elusive, particularly in renal proximal tubule epithelial cells and in the unfolded protein response (UPR) pathways in skeletal muscle cells.

Methods: Human kidney 2 (HK2) cells, a renal tubule epithelial cell line, were treated with Activin A or Transforming Growth Factor β (TGF-β) with or without, SB431542, an activin receptor-like kinase 4 receptor inhibitor, for 48 h, or treated with Activin A alone for either 6-, 18-, 24- and 48-h to investigate time-dependent effects. Western blot analysis was used to determine the expression of proteins downstream of Activin A and TGF-β signalling, and fibrotic markers. ELISA (enzyme linked immunosorbent assay) was used to quantify the concentration of Activin A present in cell culture media. C2C12 mouse-derived skeletal muscle cells were treated with Activin A for 4-h. Protein expression of UPR markers were quantified using Western blot analysis. Messenger RNA (mRNA) expression of markers for UPR and ubiquitin proteasome activation were quantified using real-time quantitative polymerase chain reaction. A SUnSET assay (surface sensing of translation assay) was used to measure changes to global protein synthesis.

Results: In HK2 cells, Activin A alone or with co-treatment with SB431542 did not alter protein abundance of downstream signalling proteins for Activin A or TGF-β or fibrotic markers, nor affect the Activin A concentration present in the media. Activin A also did not have a time-dependant effect on these parameters at 6-, 18-, 24- or 48-h. However, the protein abundance of downstream signalling proteins and fibrotic markers was significantly increased by TGF-β treatment, and Activin A concentration in the media was significantly elevated 2.35-fold compared to control treatment alone. Interestingly, TGF-β in conjunction with the inhibitor SB431542 ameliorated these increases in both protein expression and concentration of Activin A present in the media. In skeletal muscle cells, protein abundance of UPR markers was not significantly altered by Activin A treatment. However, the mRNA expression of two UPR markers, activating transcription factor 4 (ATF4) and spliced X-box binding protein 1 (XBP1s), were significantly downregulated. SUnSET assay did not indicate any significant change to global protein synthesis.

Conclusions: These findings suggest that Activin A does not induce profibrotic effects in renal proximal tubule cells at these timepoints. However, Activin A may mediate the prolonged profibrotic effects induced by TGF-β exposure, which is consistent with its role in TGF-β signalling in other renal cell types, suggesting that Activin A inhibition could ameliorate profibrotic signalling of TGF-β in proximal epithelial cells also. In skeletal muscle cells, Activin A may have an inhibitory effect on the UPR. Considering that downregulation of the UPR may also induce muscle wasting and dysfunction, this novel role of Activin A on the UPR is intriguing and worth further investigation.

Abstract: FC19

The multi-omic mosaic of spiral ganglion neuron subpopulation distributions

Lily Pearson[1], Georg von Jonqueries[1], Jennie Cederholm[1], Jeremy Pinyon[1,2], Gary Housley[1]

[1]Translational Neuroscience Facility & Department of Physiology, School of Biomedical Sciences, UNSW Sydney; [2]Charles Perkins Centre, School of Medical Sciences, University of Sydney

Hearing acuity relies on the precise organization of spiral ganglion neurons (SGN), which relay frequency-specific signals from sensory hair cells in the cochlea. SGN are divided into four subtypes: Ia, Ib, Ic, and II, based on synapse location, firing properties and molecular profile. We resolve the spatial distribution of these four populations alongside a newly identified subpopulation revealed in a transgenic reporter mouse line.

Type II SGNs drive the reflex arc of the cochlear amplifier and are marked by the type III intermediate filament Peripherin (Prph). To interrogate type II SGN function, the *Prph* promotor was used to generate a *Prph*$_p$-mCherry transgenic mouse line (Pearson et al. Sci. Reports 2025). All experiments were approved by the UNSW Animal Care & Ethics Committee, with all tissue collected from animals euthanized by pentobarbital sodium (100 mg/kg, 100 mg/ml, 29 G needle). The random integration of the *Prph*$_p$-mCherry construct was determined by nanopore sequencing and detected inside the gene encoding mGluR8 (Grm8), which is selectively expressed in type Ic SGN. This combined regulatory drive resolved a heterogeneous population of SGN, identified through colocalisation of immunopositive neurons and spatial transcriptomic profiles.

mCherry expression in the inner ear was visualised *in situ* through a CUBIC1/PEGASOS clearing protocol, alongside Lightsheet fluorescent microscopy and Imaris processing pipelines. Distributions of SGN immunolabelled for subtype markers were mapped along a 'skeletonized' helix of Rosenthal's canal within the cochlea. *The Grm8-Prph*$_p$-mCherry SGN population showed a unique basally (high frequency) biased distribution. Multiplexed *in situ* hybridization (MERSCOPE) was optimized to profile the transcriptomes of individual SGN, including the *Grm8-Prph*$_p$-mCherry population, which showed differential glutamate transporter expression. This spatially resolved multi-omic framework establishes the most comprehensive map to date of SGN populations, including highlighting a new SGN delineation, defined by *Grm8-Prph*$_p$-mCherry.

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Abstract: FC20

Genetic mapping of the morphological and proteomic landscapes of adipose tissue reveals novel ECM – adipocyte interactions.

Aaron M Lambert[1,2], Soren Madsen[1,2], David E James[1,2,3]

[1] School of Life and Environmental Sciences, University of Sydney, Camperdown, New South Wales, Australia
[2] Charles Perkins Centre, University of Sydney, Camperdown, New South Wales, Australia
[3] Faculty of Medicine and Health, University of Sydney, Camperdown, New South Wales, Australia

Aim/Introduction: The adipose tissue is a highly adaptive organ capable of expanding its energy storage capacity either via increasing adipocyte size (hypertrophy) or adipocyte number (hyperplasia). Excessive adipocyte hypertrophy causes adipose tissue inflammation and dysfunction, resulting in lipid spillover and lipotoxicity in muscle, liver, and heart. Whilst adipocyte hypertrophy is a hallmark of cardiometabolic disease, adipocyte hyperplasia on the other hand, is considered protective. The mechanisms regulating the balance between hypertrophy and hyperplasia are unclear. This project aims to clarify these mechanisms using genetic analysis and deep morphological and molecular phenotyping in Diversity Outbred (DO) mice.

Method: DO mice are produced by breeding together 5 common laboratory strains (C57BL6, AJ, 129S1, NOD, NZO), and 3 wild-derived strains (CAST, PWK, WSB) in an 8-way breeding funnel. These mice are continuously outbred and maintained in-house. DO mice capture immense genetic and phenotypic diversity that mimics that of the human population making them a powerful tool for genetic mapping. We measured the average adipocyte size of inguinal white adipose tissue (iWAT) via histological stains in 412 DO mice (tissue was removed from dead animals). In a subset of 282 of these mice, we conducted iWAT proteomics, quantifying over 6200 proteins.

Results: Genetic mapping yielded 4 adipocyte size quantitative trait loci (QTLs) and over 2700 protein QTLs (pQTLs). Each adipocyte size QTL had one or more overlapping pQTLs indicating a causal gene to protein to trait link. Further single nucleotide polymorphism (SNP) analysis identified rs46563785, a genetic variant which associated with smaller adipocytes and increased expression of the iWAT proteins arylsulfatase B (ARSB) and hexosaminidase B (HEXB). These proteins also significantly inversely correlated with average adipocyte size (ARSB: $r=-0.21$, $p<0.01$, HEXB: $r=-0.44$, $p<0.0001$). ARSB and HEXB are involved in the degradation of glycosaminoglycans (GAGs) which are highly sulphated long linear polysaccharides bound to extracellular proteoglycans. Proteoglycans maintain structural support and act as an extracellular matrix (ECM) – adipocyte signalling hub where the degree of sulphation affects binding of proteins such as growth factors.

To further characterise the relationship between ARSB, HEXB and adipose tissue GAGs, we performed glycomics in iWAT from a subset of 36 DO mice. Increased expression of ARSB, HEXB, and smaller adipocytes all significantly correlated with increased sulphation levels of heparan sulphate GAGs. Knocking down ARSB in 3T3-L1 adipocytes resulted in a 35% reduction in fibroblast growth factor 2 (FGF2) signalling which has previously been shown to play a role in adipose tissue expansion and adipocyte hyperplasia.

Conclusion: Together, these results suggest that ARSB, and HEXB play a crucial role in ECM proteoglycan remodelling influencing adipocyte size potentially via the regulation of GAG sulphation and FGF2 signalling.

Molecular Insights into Regional Retinal Physiology: A Multi-Omics Approach to Macula-Periphery Features

Azhar Dzulhadj B. Arafah[1], Xiaosuo Wang[2], Ling Zhu[1], Mark C. Gillies[1]

[1]Save Sight Institute, Faculty of Medicine and Health, The University of Sydney
[2]Cardiometabolic Medicine Group, Faculty of Medicine and Health, The University of Sydney

Background: The macula is a specialised retinal region responsible for high-resolution central vision, characterised by unique cellular composition and metabolic demands compared to the peripheral retina. These regional differences underpin the macula's susceptibility to age-related dysfunction and disease. Understanding the macula's distinct molecular physiology requires integrative profiling of proteins, metabolites, and lipids to capture the complex biochemical networks driving retinal health. Multi-omics approaches enable comprehensive characterisation of these molecular signatures, offering insight into the physiological basis of macular specialisation.

Methods: Neuroretinal punches (5 mm) from macular and peripheral regions were collected from five healthy aged human donors without signs of advanced macular pathology. Samples were analysed using LC-MS–based multi-omics profiling. Proteomics employed an untargeted data-independent acquisition (DIA) approach, and metabolomics and lipidomics were profiled with targeted multiple reaction monitoring (MRM). Principal component analysis (PCA) was conducted for each omics layer to assess regional variance. Differential expression was determined with paired t-tests. Functional interpretation included canonical pathway analysis (IPA), metabolite ontology enrichment (MetaboAnalyst), and lipid ontology enrichment (LION). Cross-omics associations were visualized using Circos plots (mixOmics) to display correlations among top discriminant features. Resulting networks were exported in GML format for graph-theoretic analysis in Cytoscape, including centrality and community detection metrics.

Results: Proteomics identified 7,564 proteins, with 330 upregulated and 197 downregulated in the macula versus the periphery. Pathway analysis revealed upregulated cholesterol biosynthesis (z-score 2.82) and downregulated rod phototransduction (z-score -2.84) in the macula. Metabolomics identified 200 metabolites, with 54 upregulated and 4 downregulated in the macula, highlighting ketone body metabolism, homocysteine degradation, and mitochondrial acetyl group transfer among upregulated metabolites. BAIBA (log2FC −1.44, $p = 0.009$) and GABA (log2FC −1.49, $p = 0.01$) were identified as the most prominent downregulated metabolites. Lipidomics identified 1,029 lipids, with 239 up- and 23 downregulated in the macula. Lipid ontology enrichment indicated macular enrichment of monounsaturated fatty acids (18 carbons) involved in membrane components versus peripheral enrichment of polyunsaturated fatty acids (>18 carbons) involved in lipid storage. At the lipid class level, ceramides (OR=3.8, FDR=1.1×10^-4) and phosphatidylcholines (OR=2.9, FDR=0.011) were significantly enriched in the macula. Circos visualization of the top 10 features per omics revealed strong positive and negative correlations between amino acids, proteins, and lipids. Network analysis identified two major modules with alanine and phenylalanine as central hubs (degree=16, betweenness centrality=0.12) and protein O95810 exhibiting high connectivity (degree=14), emphasizing their pivotal roles in mediating cross-omics interactions.

Conclusion: This multi-omics analysis reveals distinct molecular profiles between the human macula and peripheral retina, highlighting differential regulation of cholesterol metabolism, energy-related metabolites, and membrane lipids consistent with regional functional specialisation. Network analyses underscore the coordinated biochemical interactions that likely support macular physiology. These findings provide a foundational molecular map of

macular–peripheral heterogeneity, informing future physiological and pathological studies within the human retina.

Abstract: FC22

Deep learning for automated quantification of histopathological features in skeletal muscles of dysferlin-deficient mice

Lia J Munut, Irene Tsioustias, Miranda D Grounds, Erin M Lloyd

Department of Anatomy, Physiology, and Human Biology, School of Human Sciences, The University of Western Australia, Perth, Western Australia, Australia

Dysferlinopathies are a form of muscular dystrophy caused by a genetic deficiency of the protein dysferlin. They are late-onset, typically manifesting in young adults with initial wasting affecting the proximal-limb-girdle and distal limb muscles (de Tombe et al., 2011). Dysferlin-deficient skeletal muscle exhibits several histopathological features, including central myonuclei, variation in myofibre size, adipocyte accumulation, and infiltration of inflammatory cells (Grounds et al., 2014). Efficient and reproducible quantification of these features remains challenging. Manual quantification is labour-intensive; thus, semi-quantitative and subjective severity scores are common practice (Grounds et al., 2014). To our knowledge, no automated tools available currently focus on analysing haematoxylin and eosin (H&E) muscle sections to quantify the histopathological hallmarks of dysferlinopathy. Therefore, this study developed an automated image analysis workflow using open-source tools, QuPath, Cellpose, and StarDist (Bankhead et al., 2017; Schmidt et al., 2018; Stringer et al., 2021).

Deep learning models were trained on manually annotated quadriceps from 10-month-old dysferlin-deficient BLA/J mice, both untreated and dexamethasone treated for variation and due to their exacerbated pathology (Lloyd et al., 2024). Histopathology was evaluated in psoas and soleus skeletal muscles from 10-month-old control C57BL/6J and dysferlin-deficient BLA/J mice (n=8), an age when histopathology manifests in select skeletal muscles. All animal procedures were approved by the Animal Ethics and Experimentation Committee of the University of Western Australia (RA/3/100/1436). Mice were anesthetised using sodium phenobarbitone (40mg/kgBM), confirmed by the absence of the pedal withdrawal reflex. Mice were euthanised (by overdose of phenobarbitone; >160mg/kgBM) and muscles were excised and fixed in 4% paraformaldehyde, stored in 70% ethanol at 4°C, then processed for paraffin histology. Transverse sections were cut, stained with H&E, and imaged using light microscopy. Histopathological features (central myonuclei, variation in myofibre size, and inflammatory cell infiltration) were quantified three times using the automated workflow, and once manually by an independent assessor. Test-retest reliability was assessed using intraclass correlation coefficient (ICC).

Between the three independent runs of the automated workflow, reliability was excellent for all histopathological features (ICC > 0.99, 95% confidence intervals (CI) > 0.98; Koo & Li, 2016). For example, myofibre size variability (expressed as the coefficient of variation) demonstrated perfect agreement between automated runs (ICC=1.00, 95% CI= [1.00, 1.00]). However, when manual quantification was included, reliability decreased. For myofibre size variability, the ICC reduced to 0.86 (95% CI = [0.67, 0.96]) indicating a shift from excellent to good agreement (Koo & Li, 2016).

These findings demonstrate that automated quantification of dysferlin-deficient skeletal muscle is highly consistent and reproducible across repeated analyses, whereas manual scoring introduces variability. Automated methods, such as the workflow introduced here, may therefore improve the reliability of histopathological assessment in dysferlinopathy, with potential use in a wider range of muscle disorders.

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Abstract: FC23

The designer cytokine IC7Fc attenuates semaglutide-induced loss of muscle mass

Raphael Delisavvas, Steve Risis, Emma McLennan, Benoit Smeuninx, Sarah Turpin-Nolan, Mark A Febbraio

Cellular & Molecular Metabolism Laboratory, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia.

Background: Glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as semaglutide (Ozempic®), have revolutionised treatment of metabolic disease because they effectively reduce total body and fat mass, circulating lipids and blood pressure while improving glucose homeostasis[1]. Concerns have emerged, however, regarding weight loss quality, since ~1/3 of total mass loss constitutes lean mass, primarily skeletal muscle, a greater proportion than with other weight loss interventions[2]. Patients treated with GLP-1RA are often of advancing age and sarcopenia, the gradual loss of muscle mass, strength and function, is typically associated with aging. Preserving muscle mass during GLP-1RA therapy is, therefore, critical. We have previously shown that IC7Fc, an engineered IL-6 family cytokine, can protect against the loss of lean mass with caloric restriction[3]. Here, we investigated whether IC7Fc could mitigate semaglutide-induced loss of muscle mass.

Methods: C57BL/6J mice were fed a modified western diet (MWD) for 8 weeks (wk) before receiving IgGFc- control (0.5 mg/kg), semaglutide (0.2 mg/kg), IC7Fc (1 mg/kg) or co-treatment (0.2 mg/kg semaglutide + 1 mg/kg IC7Fc) via subcutaneous injection bi-weekly for 6 wk. Before and throughout treatment, body composition, food intake and oral glucose tolerance was assessed. Following the intervention period tissues were collected under 0.4% isoflurane inhalation, skeletal and cardiac muscles were assessed for markers of muscle breakdown and turnover.

Results: Mice treated with semaglutide, alone or in combination with IC7Fc, showed consistent reductions ($P<0.05$) in body weight, food intake, lean mass, and fat mass compared with IgGFc and IC7Fc treatment alone. Interestingly, lean mass was regained within 48 hours after injection, suggesting a possible dehydration effect of semaglutide. Importantly, *tibialis anterior* (weight was greater ($P<0.05$) when mice were treated with IC7Fc alone or in the co-treated group compared with semaglutide treatment alone. In ongoing experiments, we are examining the mechanisms by semaglutide reduces muscle muscle and the pathways by which IC7Fc treatment can prevent muscle mass loss.

Conclusion: IC7Fc co-administration with semaglutide partially preserves muscle mass, suggesting that IC7Fc could be a viable adjunct therapy to GLP-1RA treatment.

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Evaluating skeletal muscle dysfunction and recovery in a zymosan model of critical illness in mice

Amy J Bongetti[1], Annabel Chee[1], John HV Nguyen[1], Jin D Chung[1], Wenlan Li[1], Yasmine Ali Abdelhamid[2,3], Olav E Rooyackers[4,5], Marissa K Caldow[1], Gordon S Lynch[1].

[1]Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne; [2]Department of Critical Care, Melbourne Medical School, The University of Melbourne; [3]Intensive Care Unit, The Royal Melbourne Hospital, Victoria, Australia. [4]Perioperative Medicine and Intensive Care, Karolinska University Hospital; [5]Department of Clinical Science, Technology and Intervention, Karolinska Institutet, Stockholm, Sweden.

Critically ill patients can face extreme challenges after being admitted to the Intensive Care Unit (ICU). Aside from overcoming the illness itself, many patients must also recover from significant muscle wasting and weakness incurred during their stay in the ICU (Wang *et al*., 2020). Despite the implications of potential long-term functional deficits, few studies have investigated the underlying mechanisms. This lack of information is attributed to the high rates of attrition in clinical studies post-hospital discharge and because most research has focused on the initial disease insult, such as sepsis, rather than the consequences after resolution of life-threatening critical illness (Wilcox & Ely, 2019). To address this gap in clinical understanding, pre-clinical animal models of critical illness exhibiting muscle wasting are important for understanding the basis of functional impairments in patients. Most existing rodent models are terminal, including the caecal ligation and puncture procedure (Bongetti *et al*., 2025). Instead, this study utilised a zymosan model in mice to evaluate the recovery of hindlimb muscles and the diaphragm after wasting and weakness caused by 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 (NHMRC). C57BL/6J (15-16-week-old) male mice were allocated to control, pair-fed vehicle or zymosan treated groups. Zymosan treated mice received an *i.p.* injection of zymosan (30 mg/100 g) suspended in liquid paraffin, to induce systemic inflammation. Skeletal muscle dysfunction was assessed at day (D) 4, 7, 14 and 28, after the induction of critical illness. Whole body plethysmography was used to assess respiratory function in conscious, unrestrained mice, and these measurements were complemented by comprehensive metabolic phenotyping using a Promethion 16 metabolic cage system. At experimental endpoint, mice were anaesthetised deeply with sodium pentobarbitone (60 mg/kg) and killed via cardiac excision, with terminal collection of tissues for extensive immunohistological analyses.

Tibialis anterior (TA) muscles from zymosan treated mice exhibited atrophy and functional impairment at D4 ($P<0.01$), and D7 ($P<0.05$), with recovery of muscle mass and function at D14 compared with pair-fed, vehicle treated mice. Recovery of TA muscle mass and function was preceded by the clearance of immune cell infiltration within the muscle at D7 in zymosan treated compared with pair-fed vehicle treated mice ($CD68^+$ cells; $P<0.01$ at D4). Assessment of diaphragm muscle cross-sections from zymosan treated mice at D28 revealed smaller muscle fibres ($P<0.05$; type IIB and IIX fibres), despite evidence of inflammatory cell infiltration from D4 ($CD68^+$ cells; $P<0.01$) compared with pair-fed vehicle treated mice.

The zymosan mouse model provides important insights into the mechanisms underlying the recovery from muscle wasting and weakness after critical illness. The model represents a suitable platform for evaluating therapies to attenuate systemic inflammation associated with critical illness and restore muscle function, with relevance to better understanding and treating skeletal muscle impairments in patients after ICU discharge.

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Murine arylamine n-acetyltransferase 2 as a modulator of skeletal muscle responses in MND: Implications for disease progression and myogenesis

Melinder K Gill[1], Stephanie L Howe[2], Noor Sarakeby[1], Neville Butcher[1], Shyuan T Ngo[2,3], Rodney F Minchin[1] and Frederik J Steyn[1,3]

[1]School of Biomedical Sciences, University of Queensland, Brisbane, Australia. [2]Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane, Australia. [3]Royal Brisbane and Women's Hospital, Herston, Brisbane, Australia.

Background: Skeletal muscle dysfunction occurs early in MND and likely contributes to disease onset and progression. Improved understanding of factors that impact skeletal muscle in response to disease will help facilitate the development of therapeutics that can preserve muscle health and function in MND. Here, we investigate the role of murine n-acetyltransferase 2 (mNat2), an enzyme known for its role in mitochondrial function (Choudhury et al. 2024), as a modulator of muscle responses to disease.

Methods: We assessed the *in vivo* expression and activity of mNat2 in the muscle (gastrocnemius and tibialis anterior [TA]) of SOD1^{G93A} MND mice, and the effect of germline deletion of mNat2 on disease progression in SOD1^{G93A} mice. All animals were euthanised with Pentobarbital, 200mg/kg, i.p. Additionally, we investigated the role of mNat2 on the differentiation of murine C2C12 myoblasts, using siRNA.

Results: mNat2 expression and activity increased early and remained elevated throughout disease in the gastrocnemius and TA muscles of SOD1^{G93A} mice ($p<0.01$; n=16-24 per disease stage/sex/genotype). Germline deletion of mNat2 in SOD1^{G93A} mice worsened disease progression, as evidenced by an earlier and steeper decline in disease phenotype ($p<0.01$; n=6-22 per genotype) and faster body weight loss ($p<0.01$; n=6-22 per genotype). *In vitro*, mNat2 activity and expression increased during the differentiation of C2C12 muscle cells ($p<0.05$; n=3). Knockdown of mNat2 in myoblasts reduced C2C12 differentiation and myotube formation (*Fig 1*), with bulk sequencing revealing dysregulation of pathways related to muscle regeneration and mitochondrial function.

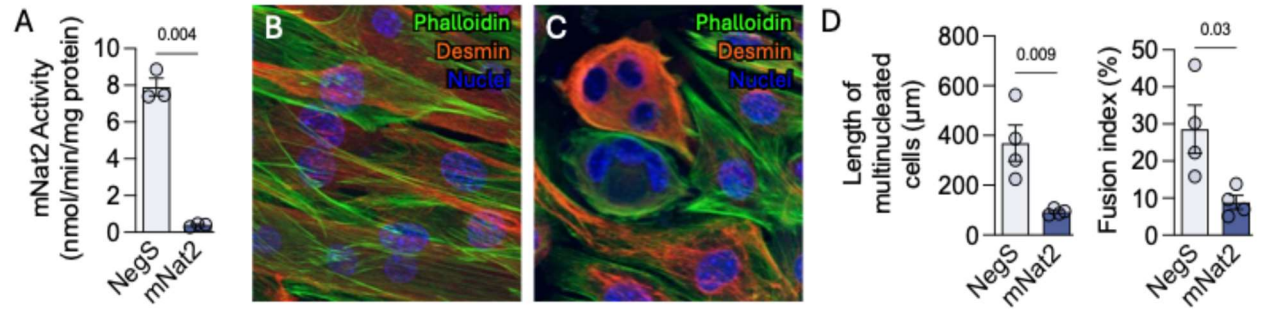

Fig 1: *mNat2-siRNA knockdown resulting in decreased mNat2 activity in C2C12 myotubes (**A**) results in impaired myoblast differentiation (representative images of 10nM negative scramble [NegS] siRNA (**B**) and 10nM mNat2-siRNA [**C**]), including a decrease in the length of multinucleated cells and fusion index (**D**). Scale bar=50µm. n=4 biological replicates.*

Conclusions: Findings suggest that an early and sustained increase in mNat2 activity in muscle may support skeletal muscle responses following the onset of the disease. *In vitro*, mNat2 was shown to contribute to myogenesis, with its knockdown leading to perturbations in pathways related to muscle regeneration and mitochondrial function. These results provide

novel evidence that mNat2 may influence skeletal muscle responses in MND; however, a direct role in muscle physiology specific to the disease remains to be fully established.

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Abstract: FC26

The integrated stress response protects muscle function in a mouse model of mitochondrial myopathy

Alexis Diaz-Vegas[1,2,*], Harry B Cutler[1,2], Kristen C Cooke[1,2], Stewart WC Masson[1,2], Søren Madsen[1,2], Oliver K Fuller[1,2], Meg Potter[1,2], Jacqueline Stöckli[1,2], Aaron Lambert[1], James Rae[4], Robert G Parton[4,5], Luke E Formosa[5], Michael T Ryan[5], Michael Lazarou[6], Louise Uoselis[6], James G Burchfield[1,2], David E James[1,2,3,*]

[1]School of Life and Environmental Sciences, University of Sydney, Camperdown, New South Wales, Australia.
[2]Charles Perkins Centre, University of Sydney, Camperdown, New South Wales, Australia.
[3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia.
[4]Centre for Microscopy and Microanalysis, The University of Queensland, Brisbane, Australia.
[5]Biochemistry and Molecular Biology, Monash University, Melbourne, Australia.
[6]Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Abstract
Mitochondrial proteotoxicity, defined as the accumulation of misfolded or unprocessed proteins, disrupts mitochondrial function and contributes to primary mitochondrial myopathies. However, the adaptive responses of skeletal muscle to this stress remain poorly understood. Here, we show that skeletal muscle–specific deletion of the mitochondrial peptidase, MIPEP, in mice recapitulates key pathological features of human MIPEP-associated myopathy, including early Complex I loss, mitochondrial swelling, cristae disruption, oxidative stress, and progressive muscle atrophy. These mitochondrial defects were accompanied by metabolic reprogramming toward glycolysis, which unexpectedly enhanced insulin responsiveness in affected muscle.

Time-resolved proteomic profiling revealed age-dependent remodelling of the muscle proteome, characterized by the misfolding of mitochondrial proteins and activation of the integrated stress response (ISR), evidenced by eIF2α phosphorylation and increased circulating levels of the stress hormones FGF21 and GDF15. Notably, ISR activation occurred before the onset of overt atrophy, suggesting an adaptive role in maintaining muscle function under proteotoxic stress. Consistent with this, pharmacological inhibition of the ISR using the eIF2B activator B2-act exacerbated muscle weakness and accelerated lean mass loss in MIPEP-deficient mice. These experiments did not require any anaesthetic, tranquilizing or muscle relaxant drugs. All tissues were collected from euthanised mice via cervical dislocation.

These findings establish the ISR as a critical protective pathway in skeletal muscle under conditions of mitochondrial proteotoxic stress. Importantly, they highlight the potential risks of broadly targeting ISR inhibition in mitochondrial myopathies, while underscoring the need to consider tissue-specific roles of this pathway in therapeutic development.

Abstract: FC27

Early biomarkers of oxidative stress and inflammation in dysferlinopathy

Georgia JM. Brown, Irene Tsioutsias, Miranda D. Grounds, Erin M. Lloyd

Department of Anatomy, Physiology, and Human Biology, School of Human Sciences, The University of Western Australia, Perth, Western Australia, Australia

Dysferlinopathies are a subgroup of muscular dystrophies caused by mutations in the *DYSF* gene, resulting in the loss of the membrane-associated protein dysferlin. The disease typically manifests in early adulthood and is characterised by progressive muscle weakness, inflammation, and eventual replacement of myofibres by adipose tissue (Grounds *et al.*, 2014). Dysferlin deficiency is known to drive muscle pathology, with current clinical monitoring tools, such as muscle biopsies and magnetic resonance imaging, offering valuable insights. However, these methods are limited by their invasiveness, cost, and availability (Warman-Chardon *et al.*, 2020). The development of blood biomarkers to monitor disease progression through a simple blood test could offer an accessible and less invasive alternative.

Previous studies have reported elevated cytokine levels in a single patient with dysferlinopathy, suggesting their potential as biomarkers of inflammation (Khaiboullina *et al.*, 2017). Additionally, albumin, the most abundant plasma protein, has been shown to undergo oxidation and serve as a blood biomarker for oxidative stress in Duchenne muscular dystrophy (Terrill *et al.*, 2024). Thus, this study aimed to identify early blood biomarkers of inflammation and oxidative stress for dysferlinopathy.

We investigated early blood biomarkers in male and female 3-month-old dysferlin-deficient BLA/J and C57BL/6J wild-type mice (n = 6). This age is of interest because it precedes the onset of overt histopathological changes in skeletal muscle. Mice were anaesthetised with Attane isoflurane (2% volume-to-volume), and blood was collected by cardiac puncture. Mice were then euthanised by cervical dislocation under anaesthesia. All animal procedures were approved by the Animal Ethics and Experimentation Committee of the University of Western Australia (2020/ET000034). Inflammatory markers were assessed by immunoblotting for interleukin (IL)-6, a multifunctional cytokine, and IL-17, a pro-inflammatory cytokine. Oxidative stress was quantified by measuring the percentage of oxidised albumin in plasma using capillary electrophoresis, a technique enabling separation of albumin in its reduced and oxidised states. Data are presented as mean ± SD.

IL-6 or IL-17 abundance did not differ between BLA/J and wild-type plasma ($p>0.05$, two-way ANOVA). Wild-type females had higher levels of IL-6 compared to wild-type males and BLA/J females ($p<0.05$, interaction, two-way ANOVA). There were no sex differences for IL-17 ($p>0.05$, two-way ANOVA). There was a significant increase in albumin oxidation in dysferlin-deficient mice. 35.6 ± 5.9% of plasma albumin was oxidised in BLA/J, compared to 25.0 ± 4.2% in wild-type controls ($p<0.001$, two-way ANOVA). There were no sex differences in the level of oxidised albumin ($p>0.05$, two-way ANOVA).

These findings show that dysferlin deficiency results in measurable systemic changes in circulating oxidative biomarkers. Although cytokine levels didn't increase at 3 months of age, the identification of elevated oxidised albumin in young BLA/J mice indicates that oxidative stress is an early feature of dysferlinopathy. This supports the potential of oxidised albumin as a minimally invasive and early biomarker for the disease. Such blood-based markers may facilitate disease monitoring, guide therapeutic evaluation, and improve the understanding of the oxidative pathways contributing to muscle pathology.

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Abstract: FC28

Evaluating a panel of plasma biomarkers of DMD in taurine-treated juvenile mdx mice

Irene Tsioutsias[1], Tony J Bakker[1], Jessica R Terrill[2], Peter G Arthur[2]

[1] School of Human Sciences, University of Western Australia, Perth WA
[2] School of Molecular Sciences, University of Western Australia, Perth WA

Duchenne muscular dystrophy (DMD) is a fatal X-linked disease characterised by muscle wasting, resulting in premature death. One of the main challenges of creating new or adjusting current treatments for DMD is the long treatment times (over a year) required to produce significant changes to clinical outcome measures. Molecular biomarkers in blood which can respond rapidly to acute changes in dystropathology, can act as earlier indicators of treatment efficacy. Such biomarkers could be used to identify treatments that are unlikely to succeed at an earlier timepoint (within a few weeks), allowing for early termination of trials. Alternatively, they could be used to more efficiently adjust regimens of current pharmaceutical and exercise treatments to minimise side effects without compromising treatment efficacy.

We have identified a panel of plasma biomarkers that have previously been shown to be elevated in dystrophy and are associated with key aspects of dystropathology. The panel includes proteins that respond to inflammation (inter-alpha-trypsin inhibitor heavy chain 4; ITIH4) oxidative stress (total thiol oxidation; AlbOx), fibrosis (osteopontin; OPN, and matrix metalloproteases 2 and 9; MMP2 and MMP9), sarcolemma instability (creatine kinase; CK) and muscle degradation (fast skeletal muscle troponin I; TNNI-2). The aim of this study was to evaluate the response of our chosen panel of biomarkers to changes in dystropathology in a model of taurine-treated juvenile dystrophic mice, which we have previously shown to prevent myonecrosis (Terrill *et al.*, 2016).

Healthy (C57) and dystrophic (mdx) mice (n = 6 - 8) were sampled at 23 days (peak myonecrosis). Juvenile mdx mice were treated with taurine (4% in soft chow) from 15 days to 23 days postnatal. Untreated mdx and C57 control mice received chow without taurine. At 23 days of age, mice were anaesthetised (2% v/v Attane isoflurane) and whole blood was collected by cardiac puncture, followed by cervical dislocation. The blood was immediately centrifuged, and plasma was removed and stored at – 80 °C until biochemical analysis. Biomarker proteins were quantified in plasma using immunoassays or capillary electrophoresis. Results were analysed by One-way ANOVA.

Untreated juvenile mdx mice exhibited altered levels of all panel biomarkers ($p < 0.05$), except OPN. The increased expression of ITIH4, AlbOx, CK, and TNNI-2 observed in untreated mdx mice was prevented in the taurine-treated mdx group ($p < 0.05$). Taurine treatment had no effect on markers of fibrosis ($p > 0.05$ for OPN, MMP9 and MMP2).

All panel biomarkers, with the exception of OPN, have so far responded as expected, with altered levels in mdx mice compared to healthy controls, and all but the fibrotic markers responding to taurine treatment. As the mice were sampled during the peak of the first necrotic event (23 days), muscles would not be exhibiting sufficient fibrosis (Giovarelli *et al.*, 2022) for taurine to have an effect, hence preventing myonecrosis was not expected to impact fibrotic markers. However, these results indicate that muscle-derived and innate immunity biomarkers are suitable for monitoring changes in acute myonecrosis and could be useful tools in tracking the effectiveness of treatments targeting myonecrosis.

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Abstract: FC29

Targeting the neonatal Fc receptor (FcRn) to improve skeletal muscle health in rheumatoid arthritis

Kristy Swiderski[1], Jessica Day[2,3], Cynthia Louis[2], Annabel Chee[1], Jennifer Trieu[1], Pranavie Vijayakumar[2], Jason Crisologo[1], Hongwei Qian[1], Paul Gregorevic[1], Ian P Wicks[2,3], and Gordon S Lynch[1]

[1]Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, Melbourne, Australia; [2]Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia; [3]Rheumatology Unit, Royal Melbourne Hospital, Melbourne, Australia.

Rheumatoid arthritis (RA) is a common chronic inflammatory disease affecting around 1% of people in Australia. While the primary pathology relates to joint inflammation and destruction, many RA patients also present with muscle atrophy early in disease, which can worsen despite intervention and persist despite resolution of the arthritis. RA patients with muscle atrophy have a 2-fold higher risk of falls, and a 10-fold higher risk of fractures, significantly reducing quality of life. There is a critical unmet need to identify novel targets that attenuate muscle atrophy in RA. Accumulation of IgG within adipose tissue has recently been implicated with poor metabolic health in ageing and obese mice, which can be attenuated by reducing or removing expression of the neonatal Fc gamma receptor (FcRn)[1,2], which recycles IgG to increase its half-life. We hypothesised that accumulation of immunoglobulin G (IgG), resulting from inflammation associated with RA, drives muscle atrophy and can be attenuated by reducing FcRn expression to lower skeletal muscle IgG accumulation.

All experiments were approved by the Animal Ethics Committee of The Walter and Eliza Hall Institute and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (NHMRC). Arthritis-related experiments utilised the K/B×N serum transfer-induced arthritis (STIA) model in which 12-week-old male C57BL/6 mice received a single intraperitoneal (*i.p.*) injection of pooled serum from arthritic K/B×N transgenic mice. After two weeks, mice were killed, and TA and gastrocnemius muscles were excised and weighed for biochemical and histological analyses. For *in vitro* experiments, C2C12 mouse myotubes were exposed to 0, 1, 10, or 100 µg/mL of purified mouse IgG or mouse serum albumin for 48 hours before being fixed and immunostained to determine the effect on myotube size. For *in vivo* FcRn knockdown experiments, tibialis anterior (TA) muscles of 12-week-old male C57BL/6 mice received intramuscular (*i.m.*) injections of adeno-associated virus serotype 6 (AAV6) containing either control shRNA (left TA) or FcRn shRNA (right TA). Four weeks later, mice were assigned as either controls or received an injection of serum to induce STIA. After two weeks, mice were killed and TA muscles excised and stored for biochemical and histological analyses.

Intramuscular IgG was increased in muscles of arthritic STIA mice, associated with reduced myofibre size. *In vitro*, C2C12 myotube size decreased after exposure to increasing concentrations of purified mouse IgG, but not albumin. Examination of the skeletal muscle phenotype in STIA mice revealed atrophy of type IIb and IIx muscle fibres in the TA muscles relative to control mice. *In vivo* knockdown of FcRn partially attenuated atrophy of IIb muscle fibres in STIA mice, resulting in an overall increase in myofibre size.

The findings suggest muscle wasting in the STIA murine model of RA may be mediated by IgG, which accumulates in skeletal muscles as a consequence of FcRn-mediated recycling. Reducing FcRn within skeletal muscle may represent a therapeutic strategy to address muscle atrophy and improve quality of life in RA patients.

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Abstract FC30

Developing approaches to screen for cardioprotective therapies for heart failure using human pluripotent stem cell derived cardiac organoids

Kevin I Watt[1,2], Henry Beetham[1], Timothy Johanssen[1], Thomas Martinez[1], Hayley L Pointer[1], Kathy Karavendzas[1], Adam T Piers[1], James W McNamara[1,2], Grace Moritz[1], Alejandro Hidalgo[1], Enzo R Porrello[1,2,3], David A Elliott[1,2,4]

[1] Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Melbourne, Australia
[2] Department of Anatomy and Physiology, University of Melbourne, Melbourne, Australia
[3] Department of Pediatrics, University of Melbourne, Melbourne, Australia
[4] Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia

Heart Failure with reduced ejection fraction (HFrEF) affects more than 40 million people globally. HFrEF is one of the poorest prognostic diseases with 5-year mortality rates of 50%. Current medical therapies provide symptomatic relief but fail to protect the heart from the progressive loss of cardiomyocytes that contributes to functional decline. For the last 50 years, heart transplantation has remained the only effective treatment in advanced disease. However, transplantation is limited by organ donor shortages contributing to prolonged periods on mechanical support increasing the risk of thrombosis and stroke. The development of an effective therapeutic that could protect cardiomyocytes and preserve heart function could delay, or potentially even avoid, the need for heart transplantation providing transformative change for patients.

A human pluripotent stem cell (PSC) expressing the mScarlett fluorescent protein was generated to visualise the Z-disc in the sarcomere of the terminally differentiated cardiomyocyte. A high-content imaging pipeline was developed to quantify Z-disc number and integrity following exposure to the anthracycline Doxorubicin; an established model of cardiotoxicity and HFrEF. Using a 30,000 chemical scaffold library developed by our team and optimised for drug-like properties, we screened for compounds that could protect human cardiomyocytes and/or Z-discs following exposure to Doxorubicin. Primary screens identified more than 1000 high confidence hits. Following secondary dose response screens in 2D cardiac monolayers, we profiled the 35 most potent scaffolds in human cardiac organoids. Of these, 3 compounds demonstrated preservation of Z-discs with associated increases in force generation.

This work demonstrates a new method towards high-content small molecule screens for cardioprotective molecules in human PSC derived cardiomyocytes. Using this approach, we have identified candidate chemical scaffolds suitable for drug development as novel first-in-class therapies for HFrEF.

Abstract: FC32

Phenytoin Inhibition Of Ryanodine Channels (RYR2) Depends On RYR2 Post-Translational Modification In Human Heart Failure

Maxine Deeb, Peter Molenaar and Kafa Walweel

Cardio-Vascular Molecular & Therapeutics Translational Research Group, Northside Clinical School of Medicine, University of Queensland, The Prince Charles Hospital, Brisbane, Australia

Cardiac output and rhythm depend on the release and the take-up of Ca^{2+} from the sarcoplasmic reticulum (SR) [1]. Excessive diastolic Ca^{2+} leak from the SR due to dysfunctional Ca^{2+} release channels (Ryanodine channels, RyR2) contributes to the formation of delayed after-depolarizations, which underlie the fatal arrhythmias [2]. Diastolic SR Ca^{2+} leak has been linked to arrhythmogenesis in both the inherited arrhythmia syndrome 'catecholaminergic polymorphic ventricular tachycardia' (CPVT) and acquired forms of heart disease such as heart failure (HF) [3]. Advanced HF fosters an environment of arrhythmogenicity from increased sympathetic nervous system tone, noradrenaline concentration and activation of β_1- and β_2-adrenoceptors (ARs) [4]. The hyperadrenergic state of HF results in leaky RyR2 channels attributable to PKA hyperphosphorylation and depletion of the stabilizing FK506 binding protein, FKBP12.6 [5]. Hence, stabilization of the RyR2 closed state during diastole, resulting in suppression of abnormal SR Ca^{2+} leak, is a promising therapeutic strategy against HF and lethal arrhythmias [6].

Phenytoin, commonly used to treat epilepsy, has shown potential as an antiarrhythmic by inhibiting diastolic Ca^{2+} leak in failing human cardiomyocytes [7]. Importantly, its inhibitory effect appears selective for RyR2 channels from failing hearts and not from healthy tissue, suggesting that its action may depend on RyR2's phosphorylation status [7]. Similar behaviour has been observed with other RyR2 modulators like dantrolene and calmodulin inhibitors [8].

This study explored phenytoin's inhibitory mechanism using single-channel recordings and Western blotting. RyR2 was isolated from β-blocked (n=4) and non-β-blocked (n=5) failing human hearts as described previously (9). RyR2s from β-blocked hearts (pooled from 4 hearts) were also stimulated with 10 μM noradrenaline for 15 min to mimic adrenergic stress. RyR2 were incorporated into lipid bilayers and the channel gating was measured at diastolic [Ca^{2+}]. Under these conditions, Phenytoin at 30 μM produced ~20% inhibitory effect within non-β-Blocked ($p=0.006$) and Stimulated β-Blocked ($p \leq 0.0001$) cohorts, where activation of RyR2 was shown in the β-Blocked cohort ($p=0.005$). The inhibition was associated with higher relative phosphorylation levels in non-β-Blocked (S2808: 84.28 ± 2.05%, S2814: 82.96 ± 2.00%) and stimulated β-Blocked (S2808: 97.49 ± 3.90%, S2814: 90.74 ± 4.29%) hearts, whereas β-Blocked hearts exhibit lower phosphorylation levels (S2808: 61.89 ± 1.78%, S2814: 62.77 ± 1.83%). To further test this dependency, RyR2s were dephosphorylated using PP1 or rephosphorylated using PKA or endogenous CaMKII. Dephosphorylation abolished phenytoin's effect in non-β-blocked hearts ($p=0.36$), while phosphorylation at S2808 and S2814 restored its inhibitory action ($p=0.0007$ and $p=0.0002$, respectively). In β-blocked hearts, phosphorylation reinstated phenytoin's inhibition by ~20% ($p=0.004$).

These findings demonstrate that phenytoin's ability to inhibit RyR2 is strongly dependent on the receptor's phosphorylation state. Thus, while phenytoin may be effective in HF patients with elevated adrenergic activity, it may be unsuitable for those receiving β-blockers, where RyR2 phosphorylation is reduced and the drug may paradoxically increase channel activity.

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A novel antihypertrophic L-type calcium channel modulator for the prevention of hypertrophic cardiomyopathy

Teagan S Er[1], Daniel Reinke[1], Alice J Francis[1], Tanya M Solomon[1], Helena M Viola[1,2], Henrietta Cserne Szappanos[1], Caitlyn M Richworth[1], Catherine Jenkins[1], Juan E Camacho Londoño[3], Padmapriya Ponnuswamy[3], Ciaran McFarlane[4], Filip Van Petegem[4], Livia C Hool[1,2].

[1]School of Human Sciences (Physiology), The University of Western Australia, Crawley, Western Australia, Australia. [2]Victor Chang Cardiac Research Institute; Darlinghurst, New South Wales, Australia. [3]CSL Innovation GmbH, Marburg, Germany. [4]Department of Biochemistry and Molecular Biology, Life Sciences Institute (Faculty of Medicine), The University of British Columbia, Vancouver, Canada.

Introduction: Hypertrophic cardiomyopathy is an autosomal dominant disease affecting 1:500 of the population and is the leading cause of sudden cardiac death in the young. Left ventricular hypertrophy, myocardial fibrosis and diastolic dysfunction are key pathogenic features of hypertrophic cardiomyopathy. At a cellular level cardiac myocytes exhibit cytoskeletal disarray, altered calcium myofilament sensitivity and a hypermetabolic state. The only Food and Drugs Administration approved drug for hypertrophic cardiomyopathy, mavacamten, is prescribed to patients with established severe obstructive hypertrophic cardiomyopathy. The lack of preventative treatments and the recent failure of mavacamten in non-obstructive hypertrophic cardiomyopathy in the ODYSSEY trial highlights the clinical need for the development of therapeutics that can both prevent hypertrophic cardiomyopathy development and display efficacy in non-obstructive hypertrophic cardiomyopathy. We have previously demonstrated that the L-type Ca^{2+} channel plays a role in the development of HCM facilitated by a structural-functional communication with mitochondria that can be regulated via the alpha interaction domain (AID) of the channel. In search of a preventative HCM therapy, we explored the efficacy of amino acid peptide variants that correspond to the AID of the cardiac L-type Ca^{2+} channel.

Methods: All animal experiments were performed in accordance with approval from The University of Western Australia Animal Ethics Committee. Competition binding assays were performed to identify AID variants which bound to the β subunit of the L-type calcium channel with high affinity. Two separate mouse models of human hypertrophic cardiomyopathy (*cTnI-G203S* and $\alpha MHC^{403/+}$) were treated with 5-10μM of the variant AID peptides or a scrambled AID peptide control for 5-weeks prior to the development of hypertrophy. Blood pressure measurements and echocardiography were carried out prior to and after 5-weeks of treatment. Following treatment, mice were anesthetised by inhalation of methoxyflurane followed by injection of pentobarbitone sodium (240mg kg-1, I.P) prior to excision of the heart. Cardiac myocytes were isolated from the hearts for patch clamp electrophysiology experiments or fluorescent experiments to measure mitochondrial function. Whole hearts were sectioned and applied with Masson Trichrome staining to assess fibrosis.

Results: Consistent with *in silico* predictions, 4 variant AID peptides bound to the β subunit with high affinity. *In vivo* treatment of both *cTnI-G203S* and $\alpha MHC^{403/+}$ mice with the AID-TAT variant peptides prevented the development of cardiac hypertrophy and fibrosis, and significantly improved contractility in the absence of alterations in L-type calcium channel conductance, blood pressure, kidney and liver function. AID-TAT variant treatment also significantly reduced the increase in flavoprotein and JC-1 fluorescence upon L-type calcium

channel activation in isolated cardiac myocytes from treated mice compared to the scrambled AID-TAT control.

Conclusions: We identified 4 variant peptides that modulate LTCC function and are effective at preventing hypertrophic cardiomyopathy in two murine models of HCM without causing negative inotropic effects or toxicity in the mice. As the mechanism of action for the peptides targets the hypermetabolic state and impaired energy metabolism evident in hypertrophic cardiomyopathy, we propose that the variant peptides will be effective for treatment of HCM caused by other gene mutations.

Abstract: FC34

Early plasma proteome alterations in genetically distinct mouse models of familial hypertrophic cardiomyopathy may reveal mechanisms of disease

Hemna Govindaraju[1], Daniel Reinke[1], Muhammad Alsherbiny[2], Esther Kristianto[2], Livia Hool[1,2]

[1] Ben Beale Laboratory in Cardiovascular Research, School of Human Sciences (Physiology), The University of Western Australia, Crawley, WA, Australia.
[2] Victor Chang Cardiac Research Institute, Sydney, NSW, Australia

Familial hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disease, is characterised by pathological thickening of the left ventricle and remains the leading cause of sudden cardiac death in the young. Mutations in genes encoding proteins of the cardiac sarcomere, the functional unit responsible for contraction, underlie most HCM cases and are often linked to earlier onset and more severe disease. However, even in carriers of pathogenic sarcomeric variants, phenotypic expression and disease severity remain highly variable and difficult to predict.

To identify circulating biomarkers that serve as indicators of underlying disease processes, we performed untargeted bottom-up DIA LC–MS/MS proteomics on enriched plasma from mouse models harbouring disease-causing mutations affecting genetically distinct sarcomeric proteins: α-myosin heavy chain (αMHC), a core component of the contractile machinery that is orthologous to human β-myosin heavy chain (βMHC); and cardiac troponin I (cTnI), which regulates contraction in response to intracellular calcium. Plasma was obtained from terminal blood collection following euthanasia by intraperitoneal injection of pentobarbitone sodium (240 mg/kg) under methoxyflurane anaesthesia, in accordance with protocols approved by The University of Western Australia Animal Ethics Committee.

Differential analysis revealed distinct sets of candidate biomarker proteins altered both prior to hypertrophy and during disease progression in heterozygous αMHC-R403Q and cTnI-G203S male mice, relative to their respective healthy controls (n = 8–13 per group). Across models, functional enrichment analysis highlighted involvement of complement cascade–associated proteins at both stages of disease, indicating early systemic reprogramming of the complement system in HCM.

This study demonstrates the utility of plasma proteomics in uncovering both conserved and mutation-specific mechanisms in HCM, with findings that require additional mechanistic validation in mice and confirmation in human cohorts. This unbiased framework supports the discovery of potential targets for disease-modifying therapies, as well as candidate biomarkers to improve risk stratification to better guide personalised monitoring and clinical decision-making.

Perfusion-dependent modulation of muscle contractility: an alternative perspective on fatigue and blood flow

Simon Green[1,2]

[1]School of Health Sciences, Western Sydney University, Sydney, Australia.
[2]Department of Cardiology, Campbelltown Hospital, Sydney, Australia.

Substantial blunting of the muscle hyperaemic response during contractions is associated with increased muscle fatigability, but the contractile effects of more subtle variations in muscle blood flow are less known and the relationship between perfusion and contractility is not established.

Data were extracted from four studies published between 1962 and 2013[2-5]. These studies employed similar techniques and protocols to explore the reversibility of effects of muscle perfusion, or perfusion pressure, on muscle contractility (force output at fixed electrical stimulus) during intermittent contractions in cat hindlimb muscles or isolated human muscles. In all studies, a stable force output was achieved with the limb positioned at heart level before perfusion or perfusion pressure was decreased in a stepwise manner (cat hindlimb studies), decreased or increased in a single step (human studies), and returned to heart level (all studies).

Perfusion (or perfusion pressure) and contractility data are expressed as a percentage of values achieved during stable, 'baseline' periods with unrestricted blood flow and muscles at the same level as the heart. The relationship between perfusion and contractility based on data from cat hindlimb and human studies is shown in Fig. 1A. All studies showed that contractility effects of reducing blood flow below 'heart level' were completely reversed by restoring perfusion to normal rates as contractions continued. Cat hindlimb studies yielded more data suited to regression analysis and the slopes of the perfusion-contractility relationship from both studies (0.91, 1.04) were close to unity. Data from human studies were consistent with this relationship, including data related to the effect of increasing perfusion pressure above pressures at 'heart level'. Data from an additional human study[1] involving voluntary contractions of the calf muscle with higher levels of muscle perfusion are also consistent with this relationship (Fig. 1 A)

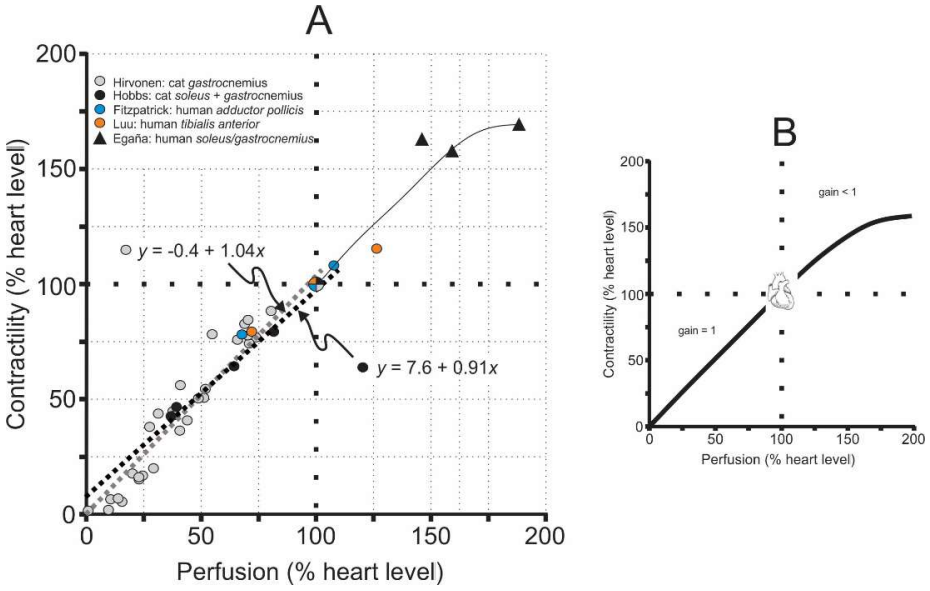

Figure 1. A. Perfusion-contractility relationship based on data from five studies cited. B. General form of the perfusion-contractility relationship inferred from data in A, showing that the gain of the relationship depends on the level of perfusion when referenced to the 'heart level'.

but suggest that the effect of perfusion on contractility plateaus at high perfusion pressure (Fig 1B).

This analysis and synthesis of evidence suggests that muscle contractility can be powerfully modulated by perfusion (gain ≈ 1) when it is close to or below levels expected when active limbs are at heart level (as in the supine position). However, the gain of the perfusion-contractility relationship appears to decline as perfusion is increased to rates well above 'heart level', as will occur for humans exercising in a more upright position.

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Abstract: FC36

Physiological and mitochondrial adaptations of overweight and obese men to high-intensity interval training in hypoxia

Zhenhuan Wang, Hiu Tung Tin, Jujiao Kuang, David Bishop, Xu Yan

Institute for Health and Sport, Victoria University, Footscray, VIC, Australia

Background: Obesity is a growing global health concern, contributing to an increased risk of metabolic and cardiovascular diseases. High-intensity interval training (HIIT) is an effective intervention for reducing body fat in overweight and obese individuals. When performed under hypoxic condition, HIIT has been shown to induce larger reductions in body fat and greater improvements in glucose tolerance, with a reduced mechanical load compared to normoxic HIIT. However, the effects of HIIT when combined with hypoxia on aerobic performance and related skeletal muscle adaptations remain controversial. As mitochondria are strongly associated with both aerobic performance and metabolic health, this study implemented an initial 4 weeks of normoxic HIIT for all participants followed by 4 weeks of HIIT combining with hypoxia, aiming to reveal the hypoxic effect when combined with HIIT on physiological and muscle mitochondrial adaptations.

Methods: Fourteen overweight or obese men (age: 31.2 ± 3.9 years, BMI: 28.0 ± 2.6 $kg \cdot m^{-2}$, $\dot{V}O_{2peak}$: 32.2 ± 4.7 $mL \cdot kg\text{-}1 \cdot min\text{-}1$) completed baseline graded exercise test (GXT) in both hypoxic (FiO_2 = 14.0%) and normoxic (FiO_2 = 20.9%) conditions. Peak power output (PPO) and the lactate threshold (LT) from hypoxic GXTs were used to prescribe training intensity. Training was performed three times per week on a cycle ergometer. Participants then repeated GXTs under hypoxic conditions for reassessment of aerobic performance and randomly assigned to either a normoxia-HIIT or a hypoxia-HIIT group (n = 7 each), matched for $\dot{V}O_2$peak per kilogram of muscle mass. Muscle biopsies were collected pre-training, after 4 weeks, and after 8 weeks. Muscle samples were analysed for mitochondrial respiratory function and content, mRNA and protein level changes.

Results: After 4 weeks of normoxic training, significant improvements were observed in $\dot{V}O_2$peak, LT, and PPO ($p < 0.05$). Mitochondrial respiration increased significantly ($p < 0.001$). Mitochondrial content, measured using Citrate Synthase (CS) activity, also increased ($p < 0.05$), while no changes were detected in the expression of mitochondrial-related genes (PGC-1α, TFAM, NRF1 and CS) or oxygen-sensing and angiogenesis related genes (HIF1-α and VEGF) ($p > 0.05$). At the protein level, no changes were found in PGC-1α, TFAM, and HIF1-α ($p > 0.05$). However, significant increases were found in protein content of mTOR, AMPKα, and AKT ($p < 0.05$), alongside a reduction in VEGF ($p < 0.05$). Following the subsequent 4-weeks training, the hypoxia-HIIT group demonstrated significant improvements in $\dot{V}O_2$peak, LT, and PPO ($p < 0.05$) compared to previous 4-week post HIIT values, whereas the normoxia-HIIT group showed no additional improvements ($p > 0.05$). No significant between-group differences were observed ($p > 0.05$). Mitochondrial respiration increased significantly only in the hypoxia-HIIT group ($p < 0.05$), while mitochondrial content, mRNA (PGC-1α, TFAM, NRF1 and CS), and protein content of mitochondrial related targets (PGC-1α and TFAM) as well as oxygen-sensing and angiogenesis-related proteins (HIF1-α and VEGF) remained unchanged in both normoxic and hypoxic training groups ($p > 0.05$).

Conclusion: Four weeks of normoxic HIIT improved aerobic performance, mitochondrial function, and CS activity, accompanied by upregulation of key signaling proteins (mTOR, AMPKα, AKT), but without significant changes in mitochondrial gene or protein expression. In the subsequent 4-week period, the significant increase in aerobic capacity and mitochondrial function were only observed in hypoxic HIIT group, with no group difference between two normoxic and hypoxic groups. This might suggest that incorporating hypoxia into HIIT may augment physiological and mitochondrial functional adaptations.

Abstract: FC37

Order-dependent effects of combined endurance and high-intensity interval training on mitochondrial adaptations in mouse skeletal muscle

Takeru Inaba, Wenxin Wang, Yasuko Oyanagi, Narumi Kaneshima, Kakeru Kogin, Yumiko Takahashi

Department of Sports Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

Background: Both moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) can induce mitochondrial adaptations that are associated with improved health and exercise performance[1,2]. Over the past decade, combined training involving MICT and HIIT has been suggested to induce greater adaptations in skeletal muscle compared to either modality alone. However, differences in exercise order studies[3] make interpretation challenging. Furthermore, the effects of exercise order in combined training on mitochondrial OXPHOS and dynamics remain unclear. This study investigated whether the order in which MICT and HIIT are performed in combined training affects mitochondrial OXPHOS and dynamics in mouse skeletal muscle.

Method: Eight-week-old male Institute of Cancer Research (ICR) mice were randomly divided into three groups: sedentary control (CON; n = 6), MICT followed by HIIT (MICT-HIIT; n = 6), and HIIT followed by MICT (HIIT-MICT; n = 7). Mice in exercise groups trained 3 weeks (3×/week) on a treadmill. The MICT protocol consisted of 30 min running at 25 m/min. HIIT was 5 bouts of 1 min at 40 m/min separated by 30 sec rest, with 15-min rest between exercises. Soleus muscles were harvested 24 h after the last bout. All mice were euthanized by bloodletting from the vena cava under isoflurane (2-4%; ISOFLURANE Inhalation Solution; VIATRIS, Tokyo, Japan) inhalation within 3 min. Protein levels of mitochondrial OXPHOS complexes and proteins regulating mitochondrial dynamics were measured.

Results: After the training period, the protein levels of mitochondrially encoded cytochrome C oxidase (MTCO1) were significantly higher in the HIIT-MICT group compared with the CON and MICT-HIIT group ($p < 0.05$). The protein level of ubiquinol-cytochrome c reductase core protein II (UQCRC2) showed no significant difference between the groups. Succinate dehydrogenase complex subunit B (SDHB) protein in the HIIT-MICT group was significantly decreased compared to CON ($p < 0.01$), but no significant differences between the exercise groups. The protein content of NADH dehydrogenase 1β subcomplex 8 (NDUFB8) and ATP synthase, H+ transporting, mitochondrial F1 complex, α subunit 1 (ATP5A) in the HIIT-MICT showed a decreasing tendency compared to CON ($p < 0.10$). In terms of proteins involved in the regulation of mitochondrial dynamics, the fusion-related proteins mitofusion 2 (MFN2) and optic atrophy 1 (OPA1) showed no significant differences among groups. The fission-related proteins in the HIIT-MICT group revealed that fission protein 1 (FIS1) was significantly higher compared with the CON and MICT-HIIT groups ($p < 0.01$), whereas dynamin-related protein 1 (DRP1) showed no difference.

Conclusion: These findings suggest that the order of combined exercise influenced mitochondrial adaptations. Changes in the levels of some mitochondrial OXPHOS subunits and proteins involved in the regulation of mitochondrial dynamics were particularly observed when HIIT was performed prior to MICT.

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Four weeks of Pycnogenol supplementation may enhance 30 second Wingate peak power output in trained cyclists

Samantha TC Kucewicz[1,2], Andrew Govus[2], Matthew Driller[2], Chris van der Poel[1,3]

[1]Department of Microbiology, Anatomy, Physiology & Pharmacology, School of Agriculture, Biomedicine & Environment, La Trobe University, Melbourne, Victoria, Australia.
[2]Sport, Performance, and Nutrition Research Group, School of Allied Health, Human Services, and Sport, La Trobe University, Melbourne, Victoria, Australia.
[3]Holsworth Biomedical Research Centre, La Trobe Rural Health School, Bendigo, VIC, Australia.

Introduction: Pycnogenol (PYC) is a (poly)phenol-rich dietary supplement extracted from French maritime pine bark, marketed predominately for its antioxidant, anti-inflammatory and blood flow modulating properties, which have led to its use as an ergogenic aid to enhance endurance performance in athletes. While previous studies have found PYC supplementation delays time to fatigue in cyclists when performing at 95% of peak power output by 15-17% (Mach et al., 2012; Bentley et al., 2012), its effects on performance in long-duration oxidative and short, high intensity exercise tasks remains unclear. The present study therefore investigated whether the effects of four weeks of PYC supplementation, compared to a placebo (PLA), on the physical performance of trained male and female cyclists.

Methods: Using a randomised, single-blind, placebo-controlled design, 20 (11 males, 8 females, mean ± SD age: 34 ± 6 years) trained cyclists performed a 6-second all out sprint, a 30-second Wingate test and 30-minute cycle test at baseline and four weeks after daily oral supplementation with either PYC (260 mg PYC) or placebo (0 mg PYC).

Results: Following four weeks of PYC supplementation, absolute peak power output (PPO) increased from 864 ± 181 W to 928 ± 164 W, whereas the placebo group showed a slight decrease from 991 ± 261 W to 985 ± 233 W. Relative PPO in the PYC group increased from 12.8 ± 2.2 W/kg to 13.8 ± 1.9 W/kg, while the placebo group remained unchanged (13.7 ± 3.0 W/kg to 13.7 ± 2.8 W/kg). A time × treatment interaction was observed for 30-second Wingate absolute PPO (parameter estimate [95% CI]: -69 W [−132, −6], $p = 0.034$) and relative PPO (−0.97 W/kg [−1.86, −0.08], $p = 0.035$). There was no time × condition interactions present for any other performance measures recorded during the 6-second anaerobic test, 30-second Wingate test and 30-minute cycle test (all $p > 0.05$).

Conclusion: In trained cyclists, 4-weeks of 260 mg of daily oral PYC supplementation resulted in enhanced 30-second Wingate peak power output compared to the PLA condition. These findings suggest PYCs' ergogenic effects are exercise intensity specific, improving glycolytic but not aerobic performance.

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Comparing the effects of intermittent and continuous exercise on core temperature

Kathleen M O'Leary[1], Adreana Koutoulas[1], Joanne N Caldwell Odgers[1]
[1]Department of Physiology, Monash University, Victoria 3800, Australia

Introduction: Maintaining a high level of aerobic fitness through regular physical activity is strongly associated with enhanced physical and mental health in older adults, and is widely recognized as a foundational strategy for the prevention and management of chronic diseases (1). However, older individuals have impaired thermoregulatory mechanisms, which is increasingly concerning given the rise in global temperatures and the frequency of extreme heat events (1,2). This places older individuals at an increased risk of heat stress, particularly during physical activity. While some studies suggest that taking breaks can help prevent overheating, others indicate that intermittent exercise may actually raise the risk of hyperthermia (3). Therefore, understanding how continuous versus intermittent exercise affects core temperature (T_c) is essential for optimizing thermoregulatory strategies in vulnerable populations. This study focused on younger adults to model healthy thermoregulatory responses which can be compared to those of older adults. The aim of this study was to investigate the effects of continuous and intermittent exercise on T_c and sweat rate in young adults to determine which type of exercise is safer in hot conditions. We hypothesised that a significant increase in T_c would be observed during continuous exercise, compared to intermittent exercise.

Methods: Eight healthy young adults (female n=4, male n=4) were recruited for this study. Informed consent was obtained, USG, and the participants clothing and nude body mass recorded. Skin thermistors, sweat patches, and a Polar heart rate (HR) monitor were attached to the participant. The participant sat in a thermoneutral (22°C) environment, before being moved to the 30°C tent where they remained seated for 30 minutes. Participants began exercise, either walking on a treadmill at 4.6km/hr continuously for 75 minutes, or at 6km/hr for 15 minutes followed by 5 minutes of seated rest, continuing until 75 minutes had elapsed where the total distance for both trials was 6km. T_c, HR, and psychophysical state were recorded every 5 minutes. Sweat patches were changed every 20 minutes during exercise and immediately weighed. Upon conclusion of exercise, participants clothing and nude body mass were recorded to determine whole-body sweat losses. Data were analysed using a repeated measures two-way ANOVA or mixed-effects analysis, followed by Tukey's multiple comparisons.

Results: No significant difference in T_c, HR, or forehead sweat patch weight were observed. The 21-40, and 61-75 minute chest sweat patches weighed significantly more in the intermittent compared to the continuous trial (0.14±0.05 versus 0.09±0.04, 0.13±0.07 versus 0.07±0.04, P≤0.05). The 0-20, 21-40, and 61-75 minute back sweat patches weighed significantly more in the intermittent compared to the continuous trial (0.09±0.03 versus 0.04±0.03, 0.16±0.04 versus 0.10±0.05, P<0.05; 0.15±0.05 versus 0.07±0.03, P<0.01) (Figure 1). The higher sweat patch weight in the intermittent trial indicates a greater level of sweating which may act to cool individuals whilst exercising in the heat. Furthermore, a lack of difference in T_c

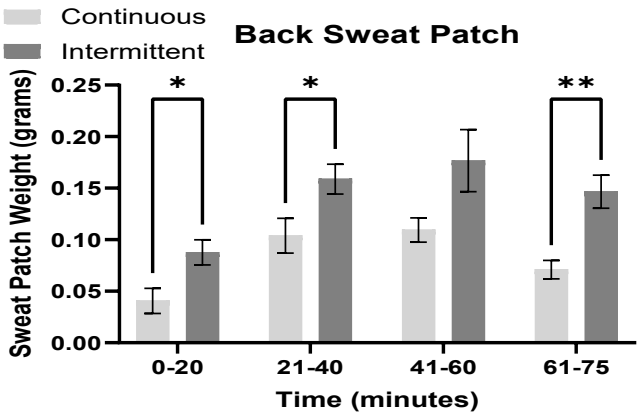

Figure 1. Back sweat patch weights following continuous and intermittent exercise (n=8). Data are presented as mean ± SEM and were analysed using a mixed-effects analysis followed by Tukey's multiple comparisons. * P<0.05, ** P<0.01

between the trials indicates that thermoregulatory mechanisms were successfully able to maintain T_c. This study was conducted on young, healthy individuals, and results may not be replicated in older individuals with impaired thermoregulatory mechanisms (2). Moreover, the study was conducted indoors in a controlled laboratory setting, whereas many individuals exercise outdoors where they are also exposed to radiant heat from the sun (2,3). Overall, this study provided a strong foundation for comparison of continuous and intermittent exercise on the impact of T_c, which can be further studied in older individuals with impaired thermoregulatory mechanisms.

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Abstract: FC41

Physiological adaptations to high altitude: longitudinal assessment of physical fitness in native and migrant university students

Nima Ouzhu[1,2], Jia Li[3,4], Jingyuan Yang[1,2], Nanhui Peng[3], Guoqing Zhai[1,2], Jujiao Kuang[3,4,5], Xu Yan[3,4,5,6]

[1]College of Education, Tibet University, Lhasa, Tibet, 85000, China. [2]Research Center for Traditional Sports and High-altitude Sports, Tibet University, Lhasa, Tibet, 85000, China. [3]Exercise and Virtual Simulation Laboratories, College of Physical Education, Southwest University, Chongqing, 400715, China. [4]Institute for Health and Sport, Victoria University, Melbourne, 3011, Australia. [5]Australian Institute for Musculoskeletal Science (AIMSS), Melbourne, 3021, Australia. [6]Department of Medicine-Western Health, The University of Melbourne, Melbourne, 3021, Australia.

High-altitude environments present distinct physiological stressors that can influence physical fitness and adaptations in young adults. This study examined the short- (migrants) and long-term effects (natives) of altitude exposure on university students' fitness profiles, based on data from the Chinese national fitness monitoring system. A total of 2,633 university students (aged 18–22 years) were assessed in Lhasa (3,650 m) and Chongqing (260 m), including high-altitude natives (n=949), high-altitude migrants (n=807), and low-altitude controls (n=877). Fitness measures, including body mass index (BMI), lung capacity, 50-m sprint (sprint), standing long jump, sit-and-reach (flexibility), sit-ups/pull-ups (strength), and 800/1000-m runs (endurance), were measured in both freshman and junior years [1].

Significant intergroup differences were found, particularly in lung capacity, sprint, and endurance. Low-altitude controls consistently outperformed both native and migrant groups, while high-altitude groups exhibited progressive declines in several performance indices over time, except for flexibility. Notably, gender-specific patterns emerged: native females demonstrated the lowest performance across multiple indicators, and regression analyses revealed an inverse "J"-shaped relationship between Physical Fitness Index (PFI) [2] and BMI, with stronger effects in males.

In summary, altitude exposure and BMI exert significant, gender-specific influences on fitness development in young adults. These findings indicate the importance of considering both environmental and anthropometric factors when designing fitness assessments and interventions for populations residing at or transitioning to high altitude.

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Associations between improvements in aerobic capacity and mitochondrial adaptations following 4 weeks of high-intensity interval training in sedentary young women

Hiu Tung Tin[1], Zhenhuan Wang[1], Itamar Levinger[1, 2, 3], Xu Yan[1, 3]

[1]Institute for Health and Sport (iHeS), Victoria University, Melbourne, Australia.
[2]University of Melbourne and the Department of Cardiology, Austin Health, Melbourne Australia
[3]Australia Institute for Musculoskeletal Sciences (AIMSS), Melbourne, Australia

Background:
High-intensity interval training (HIIT) is recognised as a time-efficient strategy to improve aerobic capacity, particularly peak oxygen uptake ($\dot{V}O_2$peak), in both healthy and clinical populations. Muscle mitochondrial adaptations, such as increased respiratory capacity and content, are often assumed to underpin these improvements. However, evidence linking individual $\dot{V}O_2$peak responsiveness after training to muscle mitochondrial changes remains inconsistent, particularly in sedentary young women. The aim of this study was to investigate whether the changes in $\dot{V}O_2$peak following four weeks of supervised HIIT are associated with concurrent changes in skeletal muscle mitochondrial respiration and citrate synthase (CS) activity.

Methods:
Sedentary young women (n = 23; age 29.7 ± 5.9 years; body mass index 25.3 ± 4.9 kg/m²) completed four weeks of supervised HIIT (3 sessions per week on a cycle ergometer). $\dot{V}O_2$peak was assessed via graded exercise testing pre- and post-intervention. Vastus lateralis muscle biopsies were obtained before and after training. High-resolution respirometry was used to quantify electron-transferring flavoprotein (ETF)-linked leak respiration (ETF L), ETF-supported oxidative phosphorylation (ETF P), complex I-supported phosphorylation (ETF+CI P), combined complex I+II-supported phosphorylation (ETF+CI+II P), and maximal electron transport capacity (ETF+CI+II E). CS activity was measured as a marker of mitochondrial content. Paired t-tests was used to compare pre- and post-values. Percent changes were calculated, and associations between changes in percentage of $\dot{V}O_2$peak and changes in percentage of mitochondrial variables were examined using spearman correlations.

Results:
$\dot{V}O_2$peak increased after HIIT (28.0 ± 6.9 to 32.7 ± 7.1 mL/kg/min/; $p < 0.05$). All measured mitochondrial respiration states demonstrated elevations compared to baseline, including ETF L, ETF P, ETF+CI P, ETF+CI+II P, and ETF+CI+II E (all $p < 0.05$). CS activity was also higher following training ($p < 0.05$), consistent with increased mitochondrial content. Despite these parallel improvements, correlation analyses revealed no associations between changes in percentage of $\dot{V}O_2$peak and changes in percentage of mitochondrial respiration: ETF L ($r = -0.15$, $p = 0.507$), ETF P ($r = 0.14$, $p = 0.552$), ETF+CI P ($r = 0.07$, $p = 0.750$), ETF+CI+II P ($r = -0.10$, $p = 0.662$), ETF+CI+II E ($r = 0.02$, $p = 0.924$), or CS activity ($r = 0.07$, $p = 0.754$).

Conclusion:
Four weeks of HIIT enhanced both aerobic capacity and skeletal muscle mitochondrial function and content in sedentary young women. However, these adaptations occurred independently, with no significant associations observed between $\dot{V}O_2$peak responsiveness and mitochondrial measures. These findings suggest that early improvements in $\dot{V}O_2$peak following short-term HIIT may be driven predominantly by non-mitochondrial factors, such as cardiovascular or haemodynamic adaptations, rather than direct mitochondrial remodelling.

Physiological effects of environmental change on physical fitness in university students

Jia Li[1,3], Nan-Hui Peng[1], Yue Zhai[1], Nima Ouzhu[2], Xu Yan[3,4,5]

[1] School of Physical Education, Southwest University, Chongqing, China
[2] Research Center for Traditional and High-altitude Sports, Tibet University, Lhasa, China
[3] Institute for Health and Sports, Victoria University, Victoria, Australia
[4] Australian Institute for Musculoskeletal Science (AIMSS), Melbourne, Australia
[5] Department of Medicine-Western Health, University of Melbourne, Melbourne, Australia

This study investigated the physiological impact of ecological migration on physical fitness in Han Chinese university students. A total of 4,671 students from Chongqing (lowland, 417 m) [1], Xinjiang (arid region, 639 m) [2], and Tibet (plateau, >3,000 m) [3] underwent standardized fitness testing. From this cohort, 2,005 students were followed longitudinally over three years and stratified into five groups: lowland Han (H_1, control), Uyghur (U_1), Tibetan (T_1), Han migrants to arid regions (HU), and Han migrants to high-altitude regions (HT), with 401 participants per group and balanced sex distribution.

A distinct ecological gradient in overall fitness was observed, with physical performance declining from lowland to arid and plateau environments. Han migrants exhibited divergent adaptation trajectories: in the arid region (HU), endurance and muscular performance improved with only minor increases in BMI, whereas in the plateau group (HT), gains were limited to endurance, with lung capacity and muscle strength remaining unchanged. Relative to native peers, migrants initially showed lower fitness but demonstrated partial recovery or compensatory improvements over time. Sex-specific responses were also evident: fitness differences between migrant and native females were smaller than those observed in males, suggesting greater stability of adaptation in females.

In conclusion, ecological migration exerts environment- and sex-specific effects on fitness development. Adaptation was more favorable in arid regions than at high altitude, highlighting the physiological challenges of hypobaric hypoxia. These findings advance understanding of human adaptability across ecological contexts and support the design of targeted health promotion strategies for students transitioning between environments.

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Abstract: FC44

High-throughput discovery and validation of metabolic health regulators

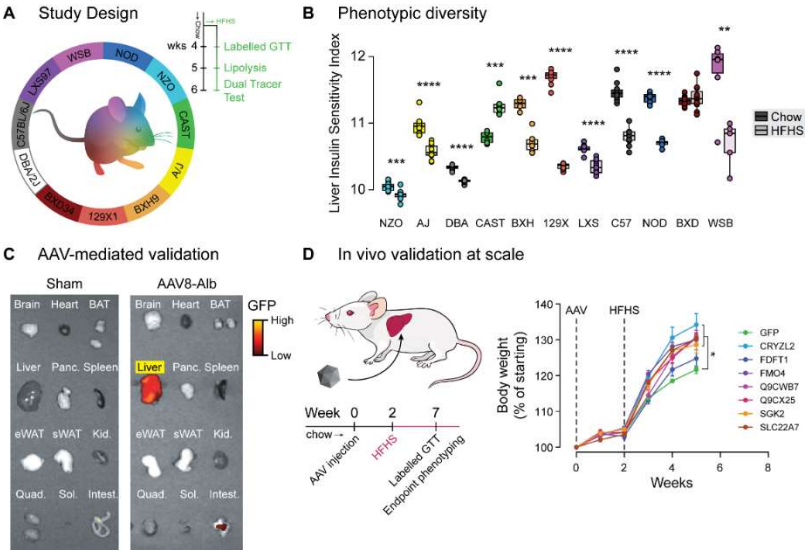
Harry B Cutler[1,2], Søren Madsen[1,2], Stewart WC Masson[1,2], David E James[1,2,3]

[1] School of Life and Environmental Sciences, University of Sydney, Camperdown, NSW, Australia
[2] Charles Perkins Centre, University of Sydney, Camperdown, NSW, Australia
[3] Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia

Understanding how genetic variation influences disease susceptibility is fundamental to the discovery of new therapies, particular for patients who fail to respond to standard treatments. Mouse models offer unique advantages for metabolic research through precise environmental control and access to deep tissues, enabling comprehensive physiological profiling across organ systems.

To identify potential new therapeutic targets, we systematically investigated diet-induced insulin resistance susceptibility across 11 genetically divergent mouse strains (Figure A). Our approach combined several innovative phenotyping techniques: a high-throughput labelled oral glucose tolerance test to assess insulin-stimulated suppression of hepatic glucose output, as well as simultaneous quantification of basal and insulin-stimulated glucose uptake in peripheral tissues using the Dual Tracer Test (Cutler, 2024). These *in vivo* glucose uptake experiments were conducted under sodium pentobarbital-induced anaesthesia (65 mg/kg administered intraperitoneally). Tissues were collected following cervical dislocation of anaesthetised mice. This approach identified considerable phenotypic diversity in terms of the response to high fat high sugar (HFHS) feeding (Figure B), providing a strong foundation for identifying molecular determinants of disease susceptibility. Proteins explaining differences in diet response between strains were identified through a deep proteomic analysis of 6 metabolically important tissues – skeletal and cardiac muscle, white and brown adipose tissues, liver and kidney – enabling us to integrate both within tissue and whole-body effects of diet. To validate hypotheses stemming from an unbiased machine-learning-based analysis of this data, we developed an Adeno-Associated Viral platform to test the physiologic effects of over-expressing specific proteins in single tissues (Figure C). These experiments are currently underway. Preliminary results are consistent with several of these proteins, including 2 uncharacterised proteins produced from open reading frames (Q9CX25 and Q9CWB7), significantly impacting the susceptibility of the mice to diet-induced obesity (Figure D). Characterising the mechanisms underpinning these effects is expected to lead to novel therapeutic strategies anchored in robust genetic evidence.

Reference: Cutler et al. (2024) *Diabetes* 73(3): 359-373.


A Study Design
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B Phenotypic diversity
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12
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NZO AJ DBA CAST BXH 129X LXS C57 NOD BXD WSB
Chow
HFHS
C AAV-mediated validation
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eWAT sWAT Kid.
Quad. Sol. Intest.
GFP
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CRYZL2
FDFT1
FMO4
Q9CWB7
Q9CX25
SGK2
SLC22A7

Abstract: FC45

The glycemic impact of whey protein ingestion in adults with type 1 diabetes

Giang M Dao[1], Sam Zhao[2], Greg M Kowalski[1], Clinton R Bruce[3], Carmel E Smart[4], Dessi P Zaharieva[5], Jennifer Ngan[2], John Apostolopoulos[2], Yee Wen Kong[2], David N O'Neal[2], Dale J Morrison[2]

[1]Institute for Physical and Activity and Nutrition, School of Medicine, Deakin University, Geelong, Australia; [2]Institute for Physical and Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, Australia; [3]Department of Medicine, The University of Melbourne, Melbourne, Victoria, Australia; [4]Department of Pediatric Diabetes and Endocrinology, John Hunter Children's Hospital, Newcastle, NSW, Australia; [5]Division of Pediatric Endocrinology, Department of Pediatrics, Stanford University, Stanford, CA, USA.

Introduction: In people with type 1 diabetes (T1D), ingestion of fast-absorbing protein potently stimulates glucagon secretion and can effectively increase endogenous glucose production. This study characterizes the glucose responses to whey protein ingestion in adults with T1D, which will inform future research into using whey protein to manage hypoglycemia in this population.

Methods: Twelve adults (7M,5F) with T1D (Age: 47.3±16.4y; BMI: 26.1±3.8kg/cm^2; Duration of diabetes: 28.1±11.5y; Mean ± SD) on insulin pumps received three interventions in random order after an overnight fast: i) water (Control), ii) low-dose protein (LOW; 0.25g/kg), iii) high-dose protein (HI; 0.5g/kg); 4 participants received a 4th intervention iv) carbohydrate (CHO; 0.25g/kg). On test days, insulin pumps were replaced with IV insulin. After 4h for subcutaneous insulin wash-out, insulin infusion rate was fixed, the test drink was given at t=0min, and blood was sampled every 10min for 3h.

Results: Peak increase and incremental area under the curve (iAUC) for blood glucose were highest for CHO compared to HI, and lowest for LOW: peak increase: [4.6 (3.4, 4.9) vs 3 (2.5, 3.3) vs 1.4 (1.1, 2) mmol/L, respectively; Median (IQR)]; iAUC: [2.3 (1.5, 3.4) vs 1.6 (1.4, 1.8) vs 0.7 (0.5, 1.2) mmol/L, respectively] (Fig. 1). Blood glucose decreased steadily throughout the sampling period for control (Fig. 1). HI compared to LOW had a higher peak increase in plasma glucagon [16 (11, 23) vs 11 (6.4, 20) pmol/L] and higher plasma glucagon iAUC [8.5 (5.6, 14) vs 4.2 (2.7, 8) pmol/L × min] (Fig. 1). Plasma glucagon remained stable for CON and was suppressed for CHO throughout the sampling period (Fig. 1). Plasma insulin was kept stable at baseline levels throughout the experimental period for all groups (Fig. 1).

Conclusion: Whey protein ingestion rapidly stimulates glucagon secretion and raises blood glucose in a dose-dependent manner in people with T1D, albeit more delayed compared to carbohydrate. This highlights a potential use of whey protein to manage hypoglycemia in people with T1D.

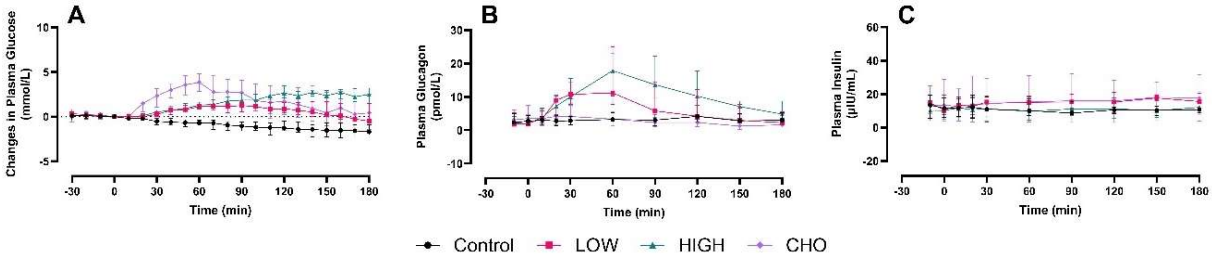

Figure 1. The glycemic and hormonal responses after an ingestion of water (Control), low-dose protein (LOW), high-dose protein (HI) and carbohydrate (CHO; n=4) in people with T1D

(n=12). (A) Changes in plasma glucose from baseline (t=0min), (B) Plasma glucagon, (C) Plasma insulin. Data presented as median and interquartile range.

Phosphatidylserine supplementation improves metabolic liver disease and glycaemic control in the presence of suppressed oxidative glucose metabolism

Li Dong[1], Sihan Lin[1], John Slavin[2], Satya Gunnam[1], Zhili Cheng[1], Shuai Nie[3], Michael G. Leeming[3], Nicholas A. Williamson[3], Magdalene K. Montgomery[1*]

[1] Department of Anatomy and Physiology, University of Melbourne, Melbourne, VIC 3010
[2] St. Vincent's Pathology, St. Vincent's Hospital, Melbourne, Victoria.
[3] Melbourne Mass Spectrometry and Proteomics Facility, Bio21 Molecular Science & Biotechnology Institute, The University of Melbourne, Melbourne, Victoria

Type 2 diabetes (T2D) and obesity are commonly accompanied by metabolic dysfunction-associated steatotic liver disease (MASLD), increasing the risk of developing metabolic dysfunction-associated steatohepatitis (MASH), fibrosis and liver cancer. The early stages of MASLD are characterized by dysfunctional lipid metabolism, including remodelling of the hepatic lipidome. In this context, reductions in hepatic phosphatidylserine (PS) have been associated with increased hepatic steatosis, inflammation and fibrosis.

In this study, we investigated the impact of dietary PS supplementation on liver function and systemic metabolic homeostasis in the MUP-uPA mouse model of MASH, including both wild-type (WT) mice with hepatic steatosis and MUP-uPA mice with severe MASH and fibrosis. Mice were fed a high-fat diet (HFD, 43% energy from fat) for 12 weeks to induce mild hepatic steatosis, followed by a subset of mice receiving HFD supplemented with 400 mg PS/kg diet for a further 12 weeks. Mice were anaesthetized using isoflurane with a maintenance rate of 2%, and all tissues were collected post-mortem.

We show that dietary PS supplementation reduces hepatic inflammation and liver fibrosis in male MUP-uPA mice. We further show that PS supplementation improves systemic glycaemic control and insulin sensitivity in both male and female mice, despite a pronounced suppression of glycolysis, glucose oxidation and glycogen breakdown in liver, skeletal muscle and/or adipose tissue. Metabolic flux analysis suggests a shift in energy substrate utilization, favouring fatty acid metabolism, particularly in skeletal muscle, while further pointing to marked improvements in mitochondrial function and overall oxidative capacity. These findings indicate that PS exerts multifaceted benefits by improving both MASH and liver fibrosis, and whole-body glucose homeostasis, independent of conventional oxidative glucose metabolism.

Our results support further investigation into dietary PS supplementation as a potential complementary strategy for MASH and glycaemic control.

Abstract: FC47

Exposure to diesel exhaust particulate matter triggers development of multi-organ insulin resistance in otherwise healthy and obese rats

Amelia Lawrence[1], Yong Song[2], Stephen Richards[1], John Burgess[1], Graeme Zosky[1, 2], Dino Premilovac[1].

[1]School of Medicine, University of Tasmania, Hobart, Australia.
[2]Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.

It is well established that insulin resistance manifests in response to diet-induced obesity. Epidemiological evidence now also points to urban air pollution exposure, and specifically diesel exhaust particulate matter (DEP), as a potential trigger for development of insulin resistance. This study aimed to determine whether acute DEP exposure alters insulin sensitivity in different organs in healthy and obese Sprague Dawley rats. To do this, six-week-old rats were maintained on a control (5% fat) or high fat diet (23% fat; HFD) for 4 weeks. At the end of the dietary intervention, rats were exposed to either vehicle or DEP (500mg) via the oropharyngeal method while under isoflurane anaesthesia (3-4%). After exposure, rats were fasted overnight and anaesthetised with pentobarbitone (85mg/kg induction; 0.06mg/kg/min maintenance). After surgery to cannulate blood vessels for intravenous infusions and arterial blood sampling, the hyperinsulinaemic euglycaemic clamp (10mU/kg/min) in combination with 2-deoxy-D-[^{14}C]-glucose and 3-[^{3}H]-D-glucose tracers was used to quantify tissue specific insulin action. Insulin's microvascular effects in skeletal muscle were assessed using the 1-methylxantine method. Acute DEP exposure did not alter fasting glucose or insulin concentrations, blood pressure or heart rate in either control or HFD-fed rats. As expected, HFD-fed rats exhibited lower whole body, skeletal muscle, adipose tissue and liver insulin sensitivity. Acute DEP exposure led to lower glucose infusion rates, reduced hepatic suppression by insulin, and reduced insulin-stimulated muscle and adipose glucose uptake in both control and HFD-rats. Insulin-stimulated microvascular blood flow in muscle was completely absent in all rats exposed to DEP. In conclusion, DEP exposure causes acute, multi-organ insulin resistance in healthy rats and further reduces insulin action in HFD-fed, insulin resistant rats. This work suggests that exposure to air pollution, such as DEP, may be an additional environmental trigger for development of insulin resistance.

The effect of inadequate sleep on glucose tolerance and mitochondrial characteristics

Hanzhe Li[1], Nicholas J Saner[1], Dale F Taylor[1], Jujiao Kuang[1], Andew Garnham[2], and David J Bishop[1]

[1]Institute for Health & Sport, Victoria University, Melbourne, Australia
[2]Institute for Physical Activity and Nutrition, Deakin University, Melbourne, Australia

Background: Inadequate sleep is common, with up to 40% of adults worldwide sleeping less than 7 h per night. Inadequate sleep not only reduces alertness but also increases the risk of developing glucose intolerance – a precursor to type 2 diabetes. While most studies have focused on severe sleep loss (<4 h/night), the impact of moderate sleep loss (<6 h/night), a pattern more representative of modern society, on glucose tolerance remains unclear. Emerging evidence suggests that alterations in mitochondrial characteristics may represent one mechanism linking sleep loss to glucose intolerance. However, further work is needed to establish a direct causal relationship. It is also unknown whether the changes induced by moderate sleep loss persist or can be reversed by a period of recovery sleep, during which adequate sleep is obtained. This study, therefore, examined whether moderate sleep loss impairs glucose tolerance and mitochondrial characteristics, and if these changes are restored by recovery sleep.

Methods: Twenty-five healthy females (24 ± 3 y; 164.1 ± 6.5 cm; 59.3 ± 8.0 kg) completed a 13-night protocol in the Victoria University Sleep Laboratory. After two baseline nights (8.5 h time in bed (TIB)), participants were randomised to either adequate sleep (AS (n = 13): 8.5 h TIB) or inadequate sleep (IS (n = 12): 6 h TIB) for seven nights, followed by four recovery nights of sleep (8.5 h TIB, both groups). Skeletal muscle biopsies were collected at baseline, after the first night of the intervention, after seven nights of the intervention, and after recovery sleep to assess changes in mitochondrial respiration, citrate synthase (CS) activity, and the proteome. Oral glucose tolerance and serum insulin concentrations were assessed through oral glucose tolerance tests (OGTTs) performed following each muscle biopsy.

Results & Conclusion: Moderate sleep loss significantly impaired glucose tolerance, with total area under the curve (AUC) increased by 9.4% after one night and 10.2% after seven nights, and incremental AUC increased by 31.4% after seven nights. Indices of insulin sensitivity were also adversely affected, with the Matsuda index decreasing by 34% and HOMA-IS decreasing by 50% after the first night. These changes were accompanied by a reduction in mitochondrial respiration (-19% after seven nights) and CS activity (-28% after the first night). By contrast, minimal changes were observed in the expression of mitochondrial proteins. Recovery sleep restored incremental glucose AUC but not total glucose AUC, metrics of insulin sensitivity (Matsuda index -25%), or mitochondrial respiration (−21%). These findings highlight altered mitochondrial characteristics as a potential mechanistic link between inadequate sleep and glucose intolerance, underscoring the importance of adequate sleep for metabolic health.

Abstract: FC49

CRISPR screening identifies APBB2 as a novel regulator of hepatic lipid metabolism

Jie Lu[1], Garron Dodd[1], Denzil Furtado[2], Michael Leeming[3], Sihan Lin[1], Zhili Cheng[1], Nicholas Willamson[3], Li Dong[1], Magdalene K Montgomery[1]

[1] Department of Anatomy and Physiology, The University of Melbourne, Melbourne, VIC, Australia
[2] Department of Chemical Engineering, The University of Melbourne, Parkville, VIC, Australia.
[3] Bio21 Molecular Science & Biotechnology Institute, Melbourne Mass Spectrometry and Proteomics Facility, The University of Melbourne, Melbourne, VIC, Australia.

Lipid metabolism dysregulation is a key feature of metabolic dysfunction-associated steatotic liver disease (MASLD), yet effective therapies targeting this pathway remain elusive. This study investigates the metabolic role of a novel protein identified from a CRISPR screen in human liver-derived HepG2 cells aimed at uncovering novel regulators of lipid metabolism: amyloid beta precursor protein binding family B member 2 (APBB2).

Mice were anesthetised with isoflurane (2-4%). All tissues were removed from dead mice. Using [14C]-based metabolic tracing, Seahorse analysis of mitochondrial function, and mass spectrometry-based lipidomics and proteomics, we show that APBB2 deletion significantly reduces mitochondrial fatty acid oxidation (70% reduction) and respiratory capacity. APBB2 knockout leads to downregulation of carnitine palmitoyltransferase 1 (CPT1) and accumulation of long-chain acyl-carnitines, supporting a block at the CPT-carnitine shuttle and downstream β-oxidation steps. In parallel, unoxidized fatty acids are rerouted into triacylglycerol (TAG) and cholesteryl ester synthesis, leading to increased lipid storage and secretion.

Untargeted proteomic and lipidomic profiling further reveal remodeling of mitochondrial cardiolipins toward longer acyl chains, together with widespread changes in TCA cycle and electron transport chain proteins, consistent with impaired oxidative metabolism. These defects extend beyond fatty acids: APBB2-deficient cells exhibit reduced glutamine oxidation, altered glucose metabolism, and compensatory upregulation of glycolytic enzymes. Liver-specific knockdown of Apbb2 in mice lowered whole-body oxygen consumption and increased the respiratory exchange ratio, suggesting a systemic shift toward carbohydrate utilisation.

Together, these findings highlight APBB2 as a novel regulator of hepatic lipid metabolism, expanding our knowledge of the genetic drivers of lipid dysregulation in MASLD.

Muscle-specific BDH1 overexpression uncovers a potential role for ketone metabolism in systemic metabolic regulation

Oliver K Fuller[1], Harry B Cutler[1], Søren Madsen[1], Stewart WC Masson[1], Kristen C Cooke[1], Dylan J Harney[1], Luke Carroll[1], Byran C Bergman[2], Zhen Yan[3], Jacqueline Stöckli[1] & David E James[1]

[1]School of Life and Environmental Sciences, The University of Sydney, Sydney, Australia. [2]School of Medicine, University of Colorado Anschutz Medical Campus, Denver, Colorado, USA. [3] Virginia Polytechnic Institute and State University, Charlottesville, Virginia, USA.

Background: Insulin action in skeletal muscle reflects the available fuel and the resulting mitochondrial redox and therefore shifts with metabolic state. Ketosis is a prime example: during fasting, sustained exercise or low-carbohydrate diets, hepatic β-oxidation increases ketone delivery; these ketones both fuel skeletal muscle and signal to influence redox and inflammation[1]. These coupled fuel-and-signal effects suggest that ketone flux can directly modulate insulin action in metabolic tissues. β-Hydroxybutyrate dehydrogenase 1 (BDH1), the metabolic enzyme that interconverts β-hydroxybutyrate and acetoacetate, therefore sits at a key control point in ketone oxidation. In support of this, work from our lab suggests that muscle BDH1 expression is markedly reduced in individuals with type 2 diabetes (T2D) and those with obesity compared with lean individuals. Moreover, trained athletes exhibit higher BDH1 expression, consistent with dynamic regulation across metabolic states. These observations raise the possibility that changes in muscle BDH1 expression contributes to insulin resistance. To test this hypothesis, we generated a muscle-specific BDH1 overexpression (OE) mouse model and assessed the impact on systemic metabolism.

Methods: *Human cohort.* Muscle biopsies were obtained from lean controls, lean athletes, obese controls, and individuals with T2D; detailed criteria and procedures have been published previously[2]. Mitochondrial proteomics was performed using Orbitrap Exploris DIA-MS with DIA-NN analysis (1% FDR). *Mouse experiments.* All procedures were approved by The University of Sydney Animal Ethics Committee. MCK-Bdh1/+ mice were chow-fed under standard housing. Exercise tolerance was assessed using a treadmill ramp protocol; ketone tolerance by intraperitoneal BHB (1.8 mg/g lean mass) with tail-vein ketone measurements; and glucose tolerance by oGTT. Blood glucose was measured with a glucometer, and plasma insulin at 0 and 15 min by ELISA. Mice were humanely euthanised using pentobarbital sodium (200 mg/kg) administered via IP injection.

Results: In a human cohort comprising four groups, broadly classified as athletes, lean, obese and T2D, BDH1 emerged as the most strongly upregulated gene when comparing athletes with participants with T2D. Moreover, its expression followed a graded decline across groups, athletes highest, then lean, obese, and lowest in T2D. To examine whether modulating BDH1 alters systemic metabolism, we generated mice with skeletal muscle–specific BDH1 OE via the MCK promoter**.** At endpoint, Bdh1/+ mice of both sexes were smaller with lower body weight than WT littermates. Overexpression reduced muscle mass but did not impair exercise capacity, with a significant main effect of genotype on distance run during a graded exercise test. Bdh1/+ mice also showed greater BHB tolerance with significantly lower KTT AUC. To assess systemic metabolism, we performed an oGTT. In males, glucose levels were similar between genotypes, although fasting insulin levels trended lower in Bdh1/+ mice. By contrast, female Bdh1/+ mice exhibited improved glucose tolerance, supported by a significant reduction in iAUC. Females exhibited improved glucose tolerance, reflected by a significant reduction in iAUC and reduced insulin at 15 min, with no change at baseline. Together, these findings point to a sex-specific effect: in males, BDH1 overexpression is linked to lower fasting insulin and a trend toward reduced HOMA-IR, while in females, improved glucose tolerance is accompanied by a significantly lower insulinogenic index (IGI), driven by reduced glucose AUC and attenuated insulin release. These preliminary data suggest muscle BDH1 influences

systemic metabolism, likely by enhancing muscle insulin sensitivity, with further studies underway to confirm this and the mechanisms underlying this, e.g. improved ROS handling.

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Feeding-regulated glycogen metabolism drives rhythmic liver protein secretion

Meltem Weger[1], Daniel Mauvoisin[2], Dominic Hoyle[1], Jingkui Wang[3], Eva Martin[2,3], James Rae[1], Charles Ferguson[1], Glynis Klinke[4], Michelle Cielesh[5], Kyle L. Macauslane[6], Mark Larance[5], Manfredo Quadroni[7], Iain Templeman[8], Jean-Philippe Walhin[8], Leonidas G. Karagounis[2,9], James Betts[8], Jonathan D. Johnston[10], Fanny Durussel[11], Dmitri Firsov[11], Stephane Fournier[12], Olivier Müller[12], Benjamin L. Schulz[6], Robert G. Parton[1], Benjamin D. Weger[1,2] and Frédéric Gachon[1,2,13,14]

[1]Institute for Molecular Bioscience, The University of Queensland; St Lucia, Australia; [2]Nestlé Research, Société des Produits Nestlé; Lausanne, Switzerland; [3]Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne; Lausanne, Switzerland; [4]Metabolomics Core Technology Platform, Center for Organismal Studies, Heidelberg University; Heidelberg, Germany; [5]Charles Perkins Centre, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney; Sydney, Australia; [6]School of Chemistry and Molecular Biosciences, The University of Queensland; St Lucia, Australia; [7]Protein Analysis Facility, University of Lausanne; Lausanne, Switzerland; [8]Department for Health, University of Bath; Bath, United Kingdom; [9]Institute of Social and Preventive Medicine, University of Bern; Bern, Switzerland; [10]Section of Chronobiology, School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey; Guildford, United Kingdom; [11]Department of Biomedical Sciences, University of Lausanne; Lausanne, Switzerland; [12]Lausanne University Center Hospital; Lausanne, Switzerland; [13]Department of Biomedicine, Aarhus University; Aarhus, Denmark; [14]Steno Diabetes Center Aarhus, Aarhus, Denmark.

Systemic energy homeostasis is essential for maintaining metabolic health and depends on inter-organ communication, with the liver acting as a central hub by secreting the majority of circulating blood proteins. Despite its importance, the detailed mechanisms underlying hepatic protein secretion and the consequences of their disruption, implicated in disease pathogenesis, remain incompletely understood. Our previously published work[1] demonstrated that protein accumulation in both mouse liver and blood is a rhythmic process, suggesting the involvement of active mechanisms in regulating hepatic protein secretion. To investigate this hypothesis further, we conducted a follow-up study using proteomic approaches in human and mouse blood, revealing that hepatic protein secretion follows a diurnal (24-hour) rhythm, regulated by food intake and the circadian clock protein BMAL1. (Note: Tissue and blood used in this study were removed from dead animals). Additional proteomic analysis of microsomal fractions from mouse livers across the day showed that rhythmic variations in the expression of secretory pathway proteins, specifically those involved in protein *N*-glycosylation and folding within the endoplasmic reticulum and Golgi apparatus, mediate this process. Subsequent *in vivo* and *in vitro* pharmacological investigations identified that eating/feeding-fasting rhythms drive diurnal hepatic protein secretion by generating glycogen-derived glycosylation substrates (glycogenolysis) and inducing physiological endoplasmic reticulum stress. Notably, we also observed that these mechanisms of diurnal protein secretion are attenuated under conditions of obesity and are influenced by genetic variants associated with Glycogen Storage Disease and Congenital Disorders of Glycosylation. Overall, our work reveals a mechanistic link between circadian, metabolic, and secretory pathways, thereby connecting nutrient intake with fundamental liver physiology and multisystemic disease.

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Abstract: FC52

Investigating transduction efficiency of myotropic adeno-associated viral vectors

Adam Hagg[1], Hugo Herron-Vellacott[1], Lidia Madrid[2], Heledd Brown-Wright[2], Kelly Walton[1], Adam Walker[2,3], Andrew Tosolini[1,4].

[1]School of Biomedical Sciences, The University of Queensland, Queensland, Australia.
[2]Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Queensland, Australia
[3]Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, NSW, Australia
[4]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Queensland, Australia.

The development of engineered recombinant adeno-associated viral (AAV) capsids with enhanced specificity and efficiency for skeletal muscle transduction has expanded the toolbox to study muscle biology in the contexts of health and disease [1,2]. Despite growing use of myotropic AAVs in preclinical and clinical settings, limited direct comparisons between engineered and naturally derived serotypes hinder efforts to identify the most effective vector, information that is likely to impact fundamental and clinical research.

Here, we compare the transduction efficiency of naturally derived and engineered myotropic variants (MyoAAV and AAVMYO) following intramuscular delivery to the tibialis anterior muscle of three-month old adult C57Bl/6 mice. For interventions requiring anaesthetic, mice received 2% vaporised isoflurane in oxygen via inhalation.

Consistent with previous reports, both engineered myotropic AAVs achieve higher transgene expression than the naturally derived capsids. In addition, we identified distinct differences in transduction profile between MyoAAV and AAVMYO.

Expanding our studies beyond the myofibre, we also developed a systemic delivery strategy using myotropic and neurotropic AAVs, enabling efficient and selective targeting of both pre- and post-synaptic compartments of the neuromuscular junction in C57Bl/6 mice.

Collectively, our findings provide a direct comparison of emerging AAV capsids in skeletal muscle and demonstrate novel strategies for cell-type specific gene delivery, with potential applications in the contexts of health and disease.

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Abstract: FC53

Advancing human models of muscle regeneration through stem cell enrichment

Melinder K Gill[1], Rodney F Minchin[1], Shyuan T Ngo[2,3] and Frederik J Steyn[1,3]

[1]School of Biomedical Sciences, University of Queensland, Brisbane, Australia. [2]Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane, Australia. [3]Royal Brisbane and Women's Hospital, Herston, Brisbane, Australia.

Background: Human muscle stem cells are essential for muscle regeneration and repair, not only under normal physiological conditions but also in the context of injury and disease. While mouse muscle stem cells, such as the immortalised C2C12 cell line, are widely used to study muscle physiology and regeneration, mouse muscle lines may not fully capture human muscle physiology. A major obstacle in developing human muscle models is the isolation of pure human muscle stem cell populations from biopsies, due to limited access to human tissues and the frequent contamination by other cell types including fibroblasts. Although a variety of isolation methods have been described, no comparative framework exists to establish a gold-standard approach for achieving high efficiency, purity, and viability of human muscle cell isolation. We systematically evaluated isolation protocols to enrich human muscle stem cells, with the goal of advancing human models to study muscle regeneration in health and disease.

Methods: We compared three isolation/enrichment methods from a vastus lateralis biopsy of a healthy donor: (i) explant culture of primary muscle tissue (Steyn et al. 2020), (ii) selective enrichment of stem cells and myoblasts using ice-cold PBS treatment (Benedetti et al. 2021), and (iii) magnetic-activated cell sorting with CD56-positive beads. The resulting myoblast populations were evaluated for their proliferative capacity and ability to undergo myogenic differentiation (*Fig 1*).

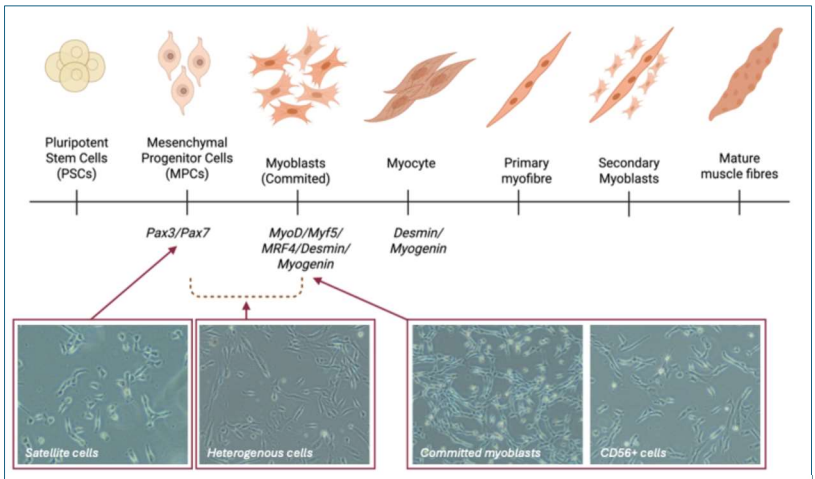

Fig 2: Schematic overview of the myogenic lineage from pluripotent stem cells to mature muscle fibers, and representative images for satellite cells, heterogeneous primary cultures, committed myoblasts, and CD56^{+}-

Results: We isolated satellite cells and activated myoblasts at distinct stages of the myogenic lineage. Compared with the routine explant isolation technique, both the pre-plating/ice-cold PBS method and CD56-positive selection yielded significantly purer cultures of satellite cells and myoblasts, as evidenced by a higher proportion of Desmin-positive cells ($p<0.05$, $n=3$) and significantly enhanced fusion index ($p<0.05$, $n=3$). Mitochondrial respiration was altered between different cell populations.

Conclusions: Our findings suggest that although cells derived from the routine explant procedure can differentiate into myotubes, their purity remains limited. The removal of contaminating cells such as fibroblasts and immune cells resulted in a higher proportion of Desmin-positive cells that formed thicker and larger myotubes. This highlights the importance

of optimised isolation strategies for improving culture purity and generating reliable human muscle stem cell models.

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Abstract: FC54

MK-8722 improves survival and attenuates systemic metabolic dysfunction in a mouse model of colon cancer

Emily S Haber[1,2], Marissa K Caldow[1,2], Annabel Chee[1,2], Jen Trieu[1,2], Amy J Bongetti[1,2], Wenlan Li[1,2], Jin D Chung[1,2], Magdalene K Montgomery[2], Justin P Hardee[1,2], Gordon S Lynch[1,2].

[1]Centre for Muscle Research, and [2]Department of Anatomy and Physiology, The University of Melbourne, Melbourne, Victoria, 3010, Australia.

Cancer cachexia affects one in four cancer patients. Metabolic dysfunction during tumour-bearing is implicated in the progressive loss of skeletal muscle and adipose tissue, increasing the risk of cancer-related mortality. Interventions to preserve or restore metabolism in cancer cachexia have broad appeal, but traditional approaches, such as exercise, may not be feasible for all patients. Exercise mimetics, especially those stimulating AMP-activated protein kinase (AMPK) to confer exercise-like adaptations, have therapeutic merit for cancer and other muscle wasting disorders. We investigated whether the pan-AMPK activator, MK-8722, that allosterically activates systemic AMPK, could rescue metabolic dysfunction in Colon-26 (C-26) tumour-bearing mice.

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). Twelve-week-old BALB/c male mice were anaesthetised (3-4% isoflurane), and the right flank shaved and then injected with C-26 cells (5×10^5 cells/mouse, *s.c.*) or with phosphate buffered saline. Mice were injected daily with MK-8722 (0, 1, 10 and 30 mg/kg body weight, *i.p*) from day 5 post-tumour inoculation. Survival and morphometric parameters were monitored and at endpoint (humane endpoint, survival; day 15, muscle function and biochemical cohorts), mice were anaesthetised (sodium pentobarbitone, 60 mg/kg, *i.p.*) for the assessment of tibialis anterior (TA) muscle function (*in situ*) and then killed by cardiac excision while anaesthetised deeply. Fatty acid uptake and oxidation, glucose oxidation and glycogen content were assessed by biochemical assay.

Chronic administration of MK-8722 improved survival in tumour-bearing mice ($P < 0.0001$). Fatigue of the TA muscle (in response to repeated intermittent stimulation) was attenuated, and muscle glycogen content was increased ($P < 0.05$). There was a shift in substrate utilisation across multiple organs, including skeletal muscle, liver, and adipose, with MK-8722 decreasing fatty acid oxidation and increasing glucose oxidation ($P < 0.05$). These changes were accompanied by a decrease in plasma fatty acids and an increase in plasma glucose ($P < 0.05$). Lipolysis and lipogenesis were both reduced in C-26 mice treated with MK-8722 ($P < 0.05$), suggesting a rescue of cancer-induced systemic metabolic dysfunction.

Together, these findings suggest high dose treatment with MK-8722 can promote survival in C-26 tumour-bearing mice. Indeed, changes in substrate availability and utilisation are likely contributing factors to the improved survival. In the absence of an effective treatment for managing cancer cachexia, MK-8722 may have standalone benefits, or serve as an adjuvant for enhancing efficacy of current cancer treatments. Future studies are warranted to investigate the efficacy of MK-8722 with chemotherapies to enhance the clinical translation of these findings.

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Abstract: FC55

A dual action tetrapeptide analogue of psychrophilic fungal origin: potent inhibitor of human nicotinic acetylcholine receptors with antinociceptive and muscle relaxant activity

Han-Shen Tae[1], Lu Lu[2,3], Cheng Ge[2,3†], Xuekai Zhang[4], Teng Pan[2,3], Zixuan Zhang[2,3], Tao Jiang[2,3], Wenqing Cai[5], Rilei Yu[2,3] and David J Adams[1]

[1]Molecular Horizons, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW, Australia. [2]Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, China. [3]Laboratory for Marine Drugs and Bioproducts, Qingdao Marine Science and Technology Center, Qingdao, China. [4]Department of Biological Engineering, College of Chemical and Biological Engineering, Shandong University of Science and Technology, Qingdao, China. [5]Shandong Academy of Pharmaceutical Sciences, Jinan, China.

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels widely expressed in muscle, as well as in the central and peripheral nervous systems. Muscle-type nAChRs exist in two developmentally distinct forms: the fetal α1β1γδ and the adult-type α1β1εδ. In contrast, non-muscle mammalian nAChRs exhibit greater subunit diversity, comprising eight α (α2–α7, α9, and α10) and three β (β2–β4) subunits that assemble into heteropentameric (e.g., α4β2, α9α10) or homopentameric receptors (e.g., α7, α9). Venom-derived peptides from marine cone snails (conotoxins), sea anemone, scorpions, and snakes act as nAChR antagonists. Several conotoxins alleviate chemotherapy-induced neuropathic pain in animal models via α9-containing nAChRs in immune cells.[1] However, pharmaceutical use of these peptides is limited by complex chemical synthesis and high production costs due to long sequences and multiple disulfide bonds. Psychrophilic fungi, adapted to extreme cold environments, produce bioactive peptides that are typically shorter, with fewer disulfide bonds, lower immunogenicity, higher solubility, and better tissue penetration than animal-derived toxins. We investigated WvVf-OCH_3, an indole-containing tetrapeptide isolated from the Antarctic psychrophilic fungus *Pseudogymnoascus sp.* HDN17-933, which was initially reported to selectively antagonise human (h) muscle-type nAChRs heterologously expressed in *Xenopus laevis* oocytes (>98% inhibition at 100 μM).[2] This peptide is notable for its alternating L/D stereochemistry and C-terminal methyl ester modification. However, the individual contributions of its residues remain undefined, and its overall bioactivity presents significant potential for optimization. In this study, using the two-electrode voltage clamp technique, a series of analogues targeting hα1β1εδ nAChRs expressed in *X. laevis* oocytes yielded two relatively potent inhibitors, WrFr-OCH_3 and WrFk-OCH_3 (half-maximal inhibitory concentration (IC_{50}) = 290 nM and 390 nM, respectively). In addition, both displayed enhanced potency and selectivity for hα9α10 nAChRs (IC_{50} = 25 nM and 71 nM, respectively). Oocytes were harvested from adult female *X. laevis* anesthetized with 1.7 mg/mL tricaine methanesulfonate (pH 7.4). Based on the superior inhibitory activity and serum stability, WrFr-OCH_3 was selected for *in vivo* evaluation. In the oxaliplatin-induced cold allodynia rat model, WrFr-OCH_3 (1 mg/kg) significantly reduced cold allodynia within 12 h of intravenous administration. The antinociceptive effect was comparable to that of gabapentin (50 mg/kg) during the first 3 h post-administration. Additionally, WrFr-OCH_3 (600 μg/kg) reduced rat forelimb muscle strength, comparable to that of rocuronium (600 μg/kg). However, WrFr-OCH_3 had a relatively slower onset of action (20 min), compared to rocuronium (5 min). Importantly, the muscle relaxant effect of WrFr-OCH_3 persisted for over 2 h compared to rocuronium (1 h). These findings identify WrFr-OCH_3 as a promising dual-activity peptide with therapeutic potential for inflammatory pain management and muscle relaxation.

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Intramuscular sex hormone concentrations in healthy males and females across the lifespan

Viktor Engman, Annabel J Critchlow, Ross M Williams, Karel Van Bellegham, Shaun Mason, Séverine Lamon

Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia

Plasma concentrations of androgens and oestrogens are moderated by sex and naturally fluctuate across the lifespan. Recent evidence suggests that sex hormones can also be synthesised locally in skeletal muscle. Previous research is however limited by small sample sizes and the use of immunoassays, which may yield unreliable results at low hormonal concentrations. To overcome these limitations, we aimed to develop a new liquid chromatography mass spectrometry-based technique to quantitate intramuscular hormone concentrations and apply it to a sample of 179 healthy males and females representing each decade of adulthood. A muscle biopsy was obtained from the vastus lateralis and snap frozen in liquid nitrogen. Deuterated internal standards for the androstenedione, dehydroepiandrosterone, dihydrotestosterone, epitestosterone, testosterone, oestrone, oestradiol, oestriol, pregnenolone, and progesterone were added to ~25mg of muscle tissue. The steroids were extracted, and the samples analysed on a Vanquish LC coupled to an Orbitrap Exploris 240. A corresponding venous blood sample was obtained at the same time and analysed using gas chromatography mass spectrometry. Eighty-three males and 96 females aged 18 – 80 years participated in this study. In plasma, oestradiol decreased across menopause in females but remained unchanged in males. Testosterone remained unchanged in females and males, but free testosterone decreased across the lifespan in the males. In the muscle, underivatised hormones yielded low signals. However, derivatisation via either hydroxylamine hydrochloride (for androgens and progestogens) or dansyl chloride (for oestrogens) increased the peak area enabling quantitation. Previous research suggests and that the intramuscular sex hormone concentrations may be more strongly associated with skeletal muscle mass and function, and that the intramuscular fraction is not proportional to the circulating hormone levels. Applying a mass spectrometry-based technique will be used to generate the first cross-sectional dataset on intramuscular sex hormone levels in adult males and females.

Abstract: FC57

Oncostatin-M is inconsistently elevated in blood following endurance exercise and does not significantly affect breast cancer cell proliferation rate *in vitro*.

Jeremy M Drake, Naomi R Werner, Lauren E Fell, Matthew J Gedye, Sian A O'Gorman, Kimberley L Way, Michelle A Keske, Glenn D Wadley.
School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia

Oncostatin-M (OSM), part of the cytokine family, is termed a "myokine" as it is secreted from skeletal muscle during exercise (1). Several pre-clinical animal models have demonstrated that OSM has an inhibitory effect on melanoma, lung, and breast cancer (1-4). However, in human breast cancer patients, elevated serum levels of OSM have been correlated with increased lymph node metastasis and reduced survival (5). Additionally, cells from invasive breast tumours express nearly six-fold more OSM than normal tissue (5). The aim of this study was to measure OSM levels in human serum following endurance exercise and determine the effect of purified OSM on breast cancer proliferation. We hypothesized that OSM would be elevated in serum post-exercise and that this would reduce the proliferation rate of breast cancer cells *in vitro*.

Methods: Serum was obtained from fasted subjects enrolled in separate clinical exercise trials at Deakin University (approved by Deakin University Human Ethics Committee - 2024/HE000191 and 2022-008) before and immediately after exercise. Serum from healthy males (n=5) and females (n=2) was obtained before and after 60 minutes of stationary cycling at 70% of calculated VO_2 peak. Serum from males (n=8) and females (n=5) with atrial fibrillation was obtained before and after completion of a maximal treadmill test (modified Bruce protocol). OSM levels in serum were analysed across all pre- and post-exercise samples using an OSM ELISA assay (ab215543, Abcam). Purified human OSM (1ng/ml) was used to treat five different breast cancer cell lines in culture. Cells were treated with OSM-supplemented culture media for 96 hours and proliferation rate was assessed using cell counting kit-8 (CCK-8) assay (Sigma-Alrich).

Results: OSM was detectable in the serum of most participants in the healthy cohort (5 out of 7), but only in a minority of the atrial fibrillation cohort (3 out of 13). Among participants with detectable OSM (n=8), post-exercise serum levels were significantly elevated ($p<0.01$). However, treatment of cell lines with purified human OSM (1 ng/ml) did not result in a significant reduction in cell proliferation. This lack of response occurred despite the treatment concentration exceeding the highest OSM levels observed in post-exercise serum (0.058 ng/ml).

Conclusion: This study aligns with previous findings from an animal model (3), which demonstrated that circulating levels of OSM can increase following endurance exercise. However, consistent with the animal data, this response appears to be variable and highly individual. Future research should investigate the effects of administering higher doses of OSM over extended periods to further explore its inhibitory action in cell culture, as reported by others (6).

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Mechanisms Contributing to Impaired Glucose Oxidation in Diabetic Cardiomyopathy

John R. Ussher[1,2,3]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada
[2]Alberta Diabetes Institute, University of Alberta, Edmonton, Alberta, Canada
[3]Cardiovascular Research Institute, University of Alberta, Edmonton, Alberta, Canada

Diastolic dysfunction is frequently present in people with type 2 diabetes (T2D), but often remains undiagnosed as routine cardiovascular screening is not common practice in people during the early stages of T2D. This diastolic dysfunction is a key feature of diabetic cardiomyopathy, which is the presence of ventricular dysfunction in the absence of coronary artery disease and/or hypertension. The myocardium in diabetes is characterized by several disturbances in energy metabolism, with one of the most prominent changes being a robust impairment in glucose oxidation (GOx). Several mechanisms contribute to this decline in myocardial GOx, including substrate competition and the Randle Cycle effect secondary to increases in myocardial fatty acid oxidation. At a molecular level, increased transcriptional activity of forkhead box O1 and peroxisome proliferator activated receptor-α can increase mRNA expression of pyruvate dehydrogenase (PDH) kinase 4, which phosphorylates and inhibits PDH, the rate-limiting enzyme of glucose oxidation. Furthermore, dysregulation of mitochondrial calcium control secondary to increased expression of the mitochondrial calcium uniporter complex inhibitory subunit, MCUb, has also been shown to impair myocardial GOx in T2D. Intriguingly, pharmacological approaches that stimulate myocardial GOx in T2D have been shown to alleviate diastolic dysfunction and improve the pathology of diabetic cardiomyopathy.

Physiological Society of New Zealand (PSNZ) 'New and Emerging Researcher Award' Winner

GLP1 receptor agonist ameliorates high blood pressure and high blood sugar in *a novel rat model of "glucotension"*

Pratik Thakkar, Aeson Chappell, Fiona McBryde, Julian Paton

[1]Department of Physiology, Manaaki Manawa – the Centre for Heart Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand.

Background and Aim: Diabetes is the fastest-growing disease in New Zealand. Most (75%) patients with T2D have high blood pressure (BP), and half of BP patients exhibit high blood glucose (BG) such a condition we call "glucotension". Glucagon-like peptide type -1 (GLP-1) has an essential role in regulating glucose homeostasis but its efficacy has not fully established in glucotension. Given the recent finding of GLP1R expression in the carotid body and hyperactivity of this organ due to higher sympathetic nerve activity (SNA) in hypertension and diabetes, we have sought to test the hypothesis that GLP1R stimulation will modulate glucotension via reducing SNA.

Methods: First time, we report the novel model of "Glucotension" induced using high-fat diet (HFD) and Streptozotocin-STZ (via Osmotic pump delivery system) in spontaneous hypertensive rats (SHR). GLP1 agonist (Exendin-4) was given (acutely & chronically) and chemoreflex testing, blood glucose, glucose tolerance (GTT), cognitive function, BP, renal SNA were assessed. Measurement of cardiac, respiratory (plethysmography) and renal function (ultrasound) were also studied.

Results: Our results showed Exendin-4 attenuates the chemoreflex evoked SNA response along with BP in HFD+STZ fed conscious SH rats ($p<0.05$). Post-drug treatment, the SHR+HFD+STZ group showed an improvement in glucose tolerance compared to respective control groups. In chronic treatment with Exendin-4, SH rats fed with HFD only also showed improvement in cognitive functions compared to pre-drug values suggesting that improved contextual memory, indicating improvement in cerebral blood flow. SHR-HFD group showed higher systolic dysfunction compared to all other groups and Exendin-4 paused this acceleration with no further decline in dysfunction ($P=0.0018$).

Conclusion: A novel model of glucotension showed cardiac dysfunction in SHRs that was ameliorated by treatment with a GLP-1 agonist. We conclude that GLP-1 agonist provides a new way to control glucotension potentially via modulating SNA.

Abstract: SS3

Sugar mishandling in diabetic heart disease

Kim M. Mellor

Department of Physiology, University of Auckland, New Zealand.

Diabetes is a significant risk factor for diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF), but limited understanding of the underlying mechanisms has hindered development of specific treatment strategies. Diabetic patients exhibit both elevated circulating and cardiac fructose levels, and fructose-induced cardiomyocyte hypertrophy and lipid accumulation has been demonstrated *in vitro*. Our studies have evaluated the therapeutic potential of targeting cardiac fructose metabolism to treat diastolic dysfunction in diabetes. Using cardiac-targeted gene therapy approaches, and pharmacological interventions, our findings demonstrate a causal role for fructose accumulation in cardiac lipotoxicity and diastolic dysfunction in diabetes. Our recent studies suggest that cardiac fructose metabolism is a potential therapeutic target for the treatment of cardiac functional deficit in diabetes.

Novel targets and therapies for cardiometabolic disease

Bianca C. Bernardo

Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia.

Heart failure is the devastating endpoint for numerous cardiovascular and metabolic diseases. Prognosis is poor, even amongst patients on optimal guideline-directed medical therapy. People with diabetes are particularly vulnerable, with a 2-4-fold increased risk of heart failure compared to those without diabetes. Many individuals with diabetes develop heart failure even in the absence of typical risk factors such as hypertension or coronary artery disease, a condition known as diabetic cardiomyopathy. The exact causes of diabetes-induced heart failure are not fully understood. Consequently, there are no therapies specifically tailored to the diabetic heart. With diabetes projected to affect over 1.3 billion people by 2050, developing targeted therapies for diabetic heart disease is a critical priority.

The pathogenesis of diabetic heart disease is multifactorial and involves a complex interplay of biological processes, including oxidative stress, chronic inflammation, dysregulated metabolic pathways, impaired insulin signalling and cardiomyocyte death. Among the molecular regulators implicated in these processes, non-coding RNAs have emerged as key players. MicroRNAs (miRNAs) and circular RNAs (circRNAs) have received significant attention due to their aberrant expression in diabetic cardiomyopathy. MiRNAs are well-established regulators of gene expression and have been explored as potential therapeutic targets for the diabetic heart. CircRNAs, a relatively newer class of non-coding RNAs characterised by their covalently closed loop structures, are increasingly recognised for their roles in cardiac physiology and pathology. They can act as miRNA sponges, regulate transcription, and interact with RNA-binding proteins, thereby influencing gene expression and cellular function. Emerging evidence suggests that circRNAs are dysregulated in diabetic cardiomyopathy and may contribute to disease progression by modulating key pathways involved in metabolism, fibrosis, and apoptosis. Their stability and tissue-specific expression profiles make them attractive candidates for biomarker development and therapeutic intervention.

In recent years, preclinical studies have explored the use of adeno-associated viral (AAV) vectors to deliver gene therapies targeting cardioprotective pathways, metabolic regulators, and glucose transport mechanisms in models of diabetic heart disease. Here, I will share insights from our recent experience using AAV-based strategies to treat the diabetic heart and highlight critical considerations for researchers embarking on new studies, including the potential integration of non-coding RNA-based approaches.

Abstract: SS5

Systems Genetics Analysis of Cardiometabolic Disease

David E James

Charles Perkins Centre, University of Sydney, Australia

Cardiometabolic disease encompasses a group of disorders linked by a common underlying factor - insulin resistance. Dissecting its molecular basis presents significant challenges due to the interplay of genetic and environmental influences and the involvement of multiple organs, including the liver, muscle, adipose tissue, and pancreas.

To tackle these complexities, we have developed a Cardiometabolic Disease Initiative using Diversity Outbred (DO) mice, a genetically diverse population that closely mimics human variation. By subjecting these mice to either a chow control diet or a Western-style diet rich in fat and carbohydrates, we have analyzed a broad range of metabolic traits.

Through tissue-specific omics analysis using euthanised mice**,** we are uncovering novel regulatory mechanisms of insulin resistance and its association with conditions such as obesity. Our findings are shedding light on causal genes and molecular pathways, paving the way for targeted therapeutic interventions in cardiometabolic disease.

Abstract: SS6

Identification of a GxE health variant that protects against metabolic disease

Jacqueline Stoeckli

Charles Perkins Centre, School of Life and Environmental Sciences, The University of Sydney, NSW, Australia

Metabolic diseases are caused by a complex interaction between genetics and environment (GxE). We utilised genetically diverse outbred mice fed two different diets to study GxE in precise detail. Mice displayed profound variation in metabolic risk factors spanning hyperinsulinemia, obesity, fasting glycemia, glucose intolerance and hepatic steatosis with ~40% of mice developing hyperinsulinemia on chow diet and ~30% remaining metabolically healthy on Western diet. By classifying mice based on metabolic health, we identified a genome wide significant quantitative trail locus associated with metabolic health, confirming that 'health' is genetically programmed and not simply the absence of disease. Using systems genetics largely involving genetic analysis of tissue specific proteomes (from euthanised mice), we pinpointed a causal metabolic health gene at the locus that encoded a metabolic enzyme. This gene is intimately involved in metabolic homeostasis and mice with reduced levels of this metabolic enzyme exhibited a trend towards improved metabolic health. This study paves the way for the discovery of further metabolic health genes, an attractive approach for identifying novel therapeutic targets.

A Systems Genetic Analysis of the Cardiac Response to Exercise in Male and Female Hybrid Mouse Lines

Brian G Drew

Molecular Metabolism & Ageing Laboratory, Baker Heart & Diabetes Institute, Melbourne, Victoria, Australia

Exercise drives beneficial myocardial cellular responses that oppose those that occur in the setting of cardiac disease, such as myocardial infarction (MI). Indeed, exercise training prior to MI is associated with reduced infarct size, improved cardiac function and greater survival following MI, in both mice and humans. However, there remains a major knowledge gap in our understanding of the cellular and molecular drivers of this exercise-induced cardioprotection. Therefore, identifying these exercise-mediated factors may provide opportunity for the development of novel therapeutic strategies to lessen the burden of the maladaptive response to cardiac disease. To investigate these pathways, we have thus engaged a systems biology analysis of up to 100 strains of both male and female genetically diverse mouse strains from the hybrid mouse diversity panel (HMDP). Multiple replicate mice from each of these strains were either exercised for 30 days or left sedentary before collection and weighing of tissues including muscle and hearts, as well as other anthropometric data (total n~1300 mice). Subsequent analysis on these data and tissues, including genomic sequence data, lipidomic analysis of blood and heart, and proteomic analysis of heart tissue has provided a rich framework that can be mined to analyse the sex specific and genetic drivers of exercise induced adaptive responses in the heart. This presentation will discuss some of the exciting data generated from this analysis.

Physiological determinants of the variability in glucose and insulin responses to glucose feeding in young adults

Christopher S. Shaw

Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC 3216.

Insulin resistance and hyperinsulinemia are closely linked with adiposity and age and precede the deterioration in glucose control that can precipitate the onset of overt type 2 diabetes. Hyperinsulinemia is often considered a compensatory response to maintain glycemic control in the presence of insulin resistance, yet an alternative view positions hyperinsulinemia as an initial defect driving insulin resistance, weight gain and subsequent manifestations of cardiometabolic disease. Over the course of our studies in young adults without obesity, we have noticed striking variability in fasting and postprandial glucose and insulin concentrations despite the absence of excess adiposity. This has prompted us to examine dysglycemia and to further characterise hyperinsulinemia in this population to gain a more detailed understanding of the origins and trajectory of cardiometabolic disease.

In a cohort of 400 young adults without obesity, we detected disturbed glucose control in one-third of individuals, and the metabolic phenotype varied depending on the type of dysglycemia. Those with impaired glucose tolerance were characterised by greater adiposity, hyperinsulinemia and deterioration in indices of insulin sensitivity. Interestingly, those with normal glucose tolerance but a 1 h glucose concentration greater than 8.6 mmol/l had an intermediary decline in insulin sensitivity without any increase in adiposity. This indicates that an initial disturbance in postprandial glucose control can occur independently of weight gain. Dysglycemia was evident without any marked change in cardiovascular risk factors, indicating that disturbed glycemic control may be one of the earliest metabolic disturbances in young adults.

We have also examined hyperinsulinemia in a population of young adults without obesity and with normal glucose tolerance. Through detailed examination of fasting and postprandial insulin responses following a standardised 2 h oral glucose tolerance test, we have found clear presentation of fasting hyperinsulinemia and a separate presentation of hyperinsulinemia that is only evident upon feeding. We have termed this phenotype isolated postprandial hyperinsulinemia (IPH). Subsequently, we have attempted to decipher the mechanisms of fasting and postprandial hyperinsulinemia. Our findings demonstrate that hyperinsulinemia is due to enhanced beta-cell sensitivity and responsivity to glucose rather than any marked change in glycemia. IPH appears as an intermediary phenotype that is less evident in response to glucose infusion compared to the fed-state, indicating a role for gut-derived factors in the exaggerated post-prandial insulin secretory response.

Collectively, these studies demonstrate that disturbances in glycemic control commonly occur in young adults without obesity and present as one of the earliest manifestations of cardiometabolic disease. Hyperinsulinemia, on the other hand, can present in either the fasted state or exclusively in the postprandial period and is caused by hypersensitivity of the beta-cell to glucose. Further work is required to understand the metabolic and cardiovascular health trajectory of the intermediary IPH phenotype. Finally, it is noteworthy to highlight the utility in examining the variability in metabolic responses in young apparently healthy adults to inform on the initial drivers of cardiometabolic disease.

Regulation of muscle regeneration by local and distant adipose tissue

Gretchen A. Meyer[1], Chang Gui[1], Jacob C. Parson[1] and Dakota R. Kamm[1]

[1]Washington University in St. Louis, St. Louis, MO 63130 (Introduced by Bradley Launikonis, University of Queensland, AUS)

Introduction: Once considered a uniform inert storage depot for energy, adipose is now recognized as a heterogeneous and dynamic collection of localized tissues, or depots, that regulate physiology through endocrine and paracrine signaling. Skeletal muscle is undoubtably a target of this signaling as we and others have shown that leptin secreted from subcutaneous "white" adipose tissue (scWAT) is required for the maintenance of muscle mass (1), myostatin secreted from "brown"/"beige" adipose tissue (BAT) controls exercise capacity (2) and yet unknown paracrine signals from intramuscular adipose tissue (IMAT) impair contraction (3). Against this background, this work explores the role of IMAT, scWAT and BAT in muscle regeneration in mice and people.

Method & Results: 13 people undergoing elective below-the-knee amputation at Washington University School of Medicine gave informed consent for the collection of muscle and scWAT from the amputated limb. IMAT was manually dissected from muscle samples under a dissecting microscope and (in parallel with scWAT), was sub-divided for histology, RNA sequencing, and adipose progenitor cell (APC) isolation. IMAT was histologically and transcriptionally different from patient-matched scWAT, with smaller adipocytes and lower normalized enrichment scores for inflammatory and immune cell pathways across participants. In participants with diagnosed type 2 diabetes (T2DM, n=7), markers of inflammatory macrophages positively correlated with HbA1c (a measure of blood glucose control) only in scWAT, suggesting that signaling from IMAT may be more dominated by adipokines than inflammatory/immune cytokines in both health and metabolic dysfunction. Indeed, many adipokines had higher expression in IMAT compared with patient-matched scWAT, both in the intact tissue and the isolated APCs. To examine the potential role for these cytokines in muscle regeneration, we constructed a culture system in which APC conditioned media was applied to myoblasts isolated from these same participants in condition-matched and -mismatched fashion: T2DM APC – T2DM myoblasts, T2DM APC – control myoblasts, control APC – T2DM myoblasts and control APC – control myoblasts. Surprisingly we found that T2DM myoblasts were uniquely sensitive to APC secreted factors – APC conditioned media decreased T2DM myoblast fusion - but the decrease was the same whether the APCs were derived from scWAT or IMAT.

We also used these samples to explore whether IMAT was a "brown", "beige" or "white" adipose depot. Neither intact IMAT or IMAT derived APCs expressed markers of BAT, even when stimulated with the browning agent isoproterenol. This is in contrast to epimuscular adipose tissue, a recognized "beige" depot in the human shoulder (4), whose APCs responded to isoproterenol treatment and increased myoblast fusion in the culture system described above. To follow up on these results, we examined mice with genetic BAT ablation (UCP1-DTA) (5) and showed that IMAT was unaffected by the genetic modification while several depots of epimuscular adipose were partially or completely ablated. To determine how loss of BAT signaling affected muscle regeneration, we induced regeneration in the tibialis anterior muscle of UCP1-DTA and littermate mice under 2% inhaled isoflurane with intramuscular injection of 50% v/v glycerol. Muscles were collected post euthanasia for flow cytometry and histology. We observed a distinct delay in regeneration in UCP1-DTA muscles characterized by a delay in fibro-adipogenic progenitor mobilization, smaller regenerating fibers and persistent expression of the embryonic isoform of myosin heavy chain. Taken together this suggests that signaling from BAT, which

includes epimuscular adipose, is important for efficient muscle regeneration. This is in contrast to IMAT, which was recently shown to impair muscle regeneration (6).

Conclusions: Adipose-muscle cross-talk is likely depot specific. IMAT, scWAT and epimuscular adipose have unique phenotypes and express/secrete unique factors. Here we show in mice and people that IMAT, like scWAT is a “white” fat, but its unique anatomical location and transcriptional profile may cause it to uniquely affect muscle regeneration. Epimuscular adipose, on the other hand, is a “beige” fat which may support regeneration. Future studies in human cells and adipose transplant mouse models will be key to defining the relevant signals.

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Modulating myogenesis using natural bioactive compounds

Marissa K. Caldow, Kristy Swiderski, John H. V. Nguyen, Kyla H. Fritz, Emily S. Haber, Gordon S. Lynch

Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, Victoria, 3010, Australia

Skeletal muscle has an impressive capacity to remodel after stress or injury. This process is intrinsically regulated at key stages of muscle cell development ('myogenesis') to ensure successful remodelling and repair. The process can become dysregulated during different diseases or be manipulated extrinsically through pharmacological or nutritional interventions.

Nutritional supplements, specific amino acids and plant-derived bioactives, can exert effects on muscle cells *in vitro* and *in vivo*, by modulating signalling pathways controlling muscle development, inflammation and cell metabolism. It is therefore possible that specific nutrients may favourably manipulate myogenesis and muscle remodelling, with therapeutic potential for skeletal muscle injuries or muscle-degenerative diseases (Kunkel et al., 2011; Moro et al. 2016). We have shown that single amino acid feeding can protect muscle cells *in vitro* from external stressors such as inflammation and caloric restriction, and promote protein synthesis (Ham et al., 2014; 2015; 2016; Caldow et al., 2019).

Another important application of this approach relates to the optimisation of myogenesis and maturation of muscle cell phenotype, events crucial for the scaled manufacture of cultured meat and seafood. Modulating nutrients in serum to promote maturation or 'nutritive steering' is a novel approach for growing muscle to scale and overcoming one of the major challenges of cellular agriculture.

Using cultured murine skeletal muscle cells, we have examined whether muscle cell maturation can be manipulated, during specific stages of cellular growth. During proliferation and from the onset of differentiation, we have investigated plant-derived bioactives shown previously to promote hypertrophy of mature myotubes. Our findings suggest that although these compounds can confer a similar phenotype at one developmental stage, they differentially affect cell proliferation, maturation and growth when myoblasts are supplemented from the earliest stages of myogenesis. The dynamic movement of maturing myotubes, measured via live cell imaging, is also influenced by continual exposure to different compounds during differentiation.

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Muscling into Reproduction: Emerging evidence for myokine influences on reproductive systems

Adam Hagg[1], Hugo Herron-Vellacott[1], Ella Hockam[1], Daniel J Bernard[2,3] and Kelly L Walton[1,4]

[1]School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia
[2]Department of Pharmacology and Therapeutics, McGill University, Montréal, QC H3G 1Y6, Canada
[3]Department of Anatomy and Cell Biology, McGill University, Montréal, QC H3G 1Y6, Canada
[4]Department of Physiology, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Reproduction is one of the most energetically demanding processes in female mammals and therefore, highly dependent on metabolic condition. In multiple species, marked shifts in body weight and composition are associated with reduced fecundity (oocyte/egg or embryo production/quality) and fertility (live births). Reproductive function is thought to be suppressed under metabolic extremes to conserve energy for vital organs and prevent embryo development in unfavourable conditions. Until now it was thought that fat/adiposity and adipokines were the major metabolic influencers of reproductive potential. However, exciting new evidence supports that skeletal muscle secreted proteins/peptides (myokines) are major regulators of female reproductive system that have been overlooked. Our interdisciplinary reproduction and skeletal muscle physiology research team seeks to understand how skeletal muscle adaptation influences myokine-mediated actions in reproduction. We have recently established that metabolic stress induced by graded calorie restriction or endurance exercise in female mice, induces changes in the muscle secretome, influencing reproductive systems and hormone flux. Ongoing proteomic analyses seek to identify those myokines with influential activities on female reproduction. The outcomes of these studies have potential implications for the management of sub/infertility, delayed puberty onset, and gonadal dysfunction in eating/feeding disorders and energy deficiencies in sport.

Ca^{2+} regulates the acute response to sprint interval exercise in human muscle

Aldo Meizoso-Huesca[1], Quynh Phan[1], Cedric R Lamboley[1], Daniel P Singh[1], Stefan Wette[2], Jeff Coombes[3], Robyn M Murphy[2], and Bradley S Launikonis[1]

[1]*School of Biomedical Sciences, The University of Queensland, Brisbane.*

[2]*La Trobe Institute for Molecular Science, La Trobe University, Melbourne.*

[3]*School of Human Movement and Nutrition, The University of Queensland, Brisbane.*

A single session of high intensity interval training (HIIT) effects the resting muscle and underlies its adaptation to the exercise. HIIT leads to mitochondrial biogenesis and an increase in speed-endurance capacity. Up until now, it has been suggested that Ryanodine Receptor (RyR) Ca^{2+} leak is a major change post-intense exercise. Several novel functional assays were performed on freshly biopsied human muscle before and after a single session of intense exercise to track changes in the muscle that are triggered post-exercise. RyR Ca^{2+} leak was demonstrated post-exercise and associated changes in Ca^{2+} distribution and Ca^{2+} movements across the plasma/tubular (t-) system membrane were shown. New assays targeted at Plasma Membrane Calcium-Transporting ATPase (PMCA) and Sarco/Endoplasmic Reticulum Calcium ATPase (SERCA) activity in the resting muscle were developed and showed critical roles in the raised rate of oxidative phosphorylation of the resting muscle through changes in Ca^{2+} handling and response to reactive oxygen species post-intense exercise.

An epilepsy or a developmental disorder? It depends on $GABA_A$ receptor function.

Nathan L Absalom

School of Science, Western Sydney University, NSW, Australia

Genetic epilepsies are a heterogenous mix of disorders with the common clinical feature of recurrent seizures. Next generation sequencing has unearthed an enormous number of genes associated with epilepsy, over 900 at last count. Included in these genes are *GABRB2* and *GABRB3* genes that encode for the β2 and β3 subunits of the γ-aminobutyric acid type A ($GABA_A$) receptors that mediate neuronal inhibition. Variants in these genes lead to a wide spectrum of clinical outcomes including different seizure severity and types, a range of intellectual disabilities, motor dysfunction, movement disorders and have different responses to drugs.

To understand why genetic variants in these genes result in specific clinical outcomes, we have correlated specific biophysical changes in receptor function to phenotypes. Generally speaking, fundamental biophysical process understood from decades of basic structural and functional knowledge underlie the clinical features seen in individuals with $GABA_A$ receptor variants. Our hypothesis is that by quantifying the change in biophysical changes, including GABA sensitivity, structural location or desensitization process, clinical outcomes can be predicted from an early age.

$GABA_A$ receptors are an efficient machine that converts binding of the neurotransmitter GABA to chloride conductance across a neuronal membrane. Our correlations have demonstrated that GABA sensitivity is the key variable in the clinical phenotype, with distinct syndromes caused by loss or gain of function. The structural location of variants also plays a key role in the phenotype. As the receptor is activated by key conformational changes initiated by GABA binding and coupled to the ion channel pore, and the distance from the residues to the activation pathway is accurate predictor of variant pathogenicity, and the distance along the pathway is associated with a loss or gain of function. Other biophysical attributes, chiefly desensitization, play a minor role in moderating the phenotype.

However, the tight correlations between changes in variant function and clinical outcomes only extend so far. Seizure syndromes and severity are still variable at individuals with a gain of function variant. However, intellectual disability and motor dysfunction are well correlated with functional change and the age of seizure onset, a key marker of severity, such that the level of motor dysfunction can be predicted with considerable accuracy just from the functional change of the receptor and the age of onset. This consistency suggests that $GABA_A$ receptor variants lead primarily to a neurodevelopmental disorder with seizures a symptom, rather than an epilepsy phenotype with co-morbidities of intellectual disability and motor dysfunction. To properly treat the disorder, precision medicine strategies that target the cause of the disorder and measure these underlying primary clinical outcomes are required.

Abstract: SS14

Channelling uncertainty: a functional approach to assess the significance of human *CACNA1G* variants

Rocio K. Finol-Urdaneta[1,2], Chek-Ying Tan[3], Neven Maksemous[4], Joanne G. Ma[3] Lyn R. Griffiths[4], David J. Adams[1,2], Jamie I Vandenberg[3,5], Chai-Ann Ng[3,5]

[1]School of Medicine and Health; Medical, Indigenous and Health Sciences, University of Wollongong, Wollongong, NSW, Australia.
[2]Molecular Horizons, University of Wollongong, Wollongong, NSW, Australia.
[3]Mark Cowley Lidwill Research Program in Cardiac Electrophysiology, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia.
[4]Genomics Research Centre, The Centre for Genomics and Personalised Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia.
[5]School of Clinical Medicine, University of New South Wales Sydney, Darlinghurst, NSW, Australia.

Background: The classification of *CACNA1G* variants of uncertain significance (VUS) presents a major challenge in clinical genomics, particularly in the diagnosis of neurodevelopmental and epileptic disorders. The Cav3.1 T-type calcium channel encoded by *CACNA1G* is critical for neuronal excitability and rhythmic firing. Functional data are increasingly recognised as essential for variant interpretation under ACMG/ClinGen guidelines, yet scalable and quantitative approaches remain limited.

Methods: A calibrated, high-throughput functional assay was developed using automated patch-clamp electrophysiology to assess the biophysical properties of Cav3.1 variants. A reference set of 41 variants, 25 benign/likely benign (B/LB) and 16 pathogenic/likely pathogenic (P/LP), was systematically profiled. Key metrics included normalized current density (CD), voltage-dependence of activation and inactivation, and deactivation kinetics (τdeact). Statistical comparisons were performed to identify parameters that best distinguished variant classes. Five VUS were subsequently evaluated using the same pipeline.

Results: Normalized current density and deactivation kinetics emerged as the most reliable and discriminatory metrics. CD alone distinguished over 80% of P/LP variants, which exhibited significantly reduced current amplitudes compared to B/LB controls ($p < 0.001$). τdeact provided complementary insight, identifying gating abnormalities in variants with normal CD. When applied to five VUS, two variants, R186Q and R1394Q, demonstrated functional profiles consistent with pathogenicity, including reduced CD and altered τdeact, mechanistically associated with voltage-sensor dysfunction. The remaining three VUS aligned with benign reference distributions across all metrics.

Conclusion: This study establishes a robust and scalable framework for the functional classification of *CACNA1G* variants. By integrating electrophysiological data into variant interpretation, the assay provides a quantitative basis for reclassifying VUS, thereby reducing diagnostic uncertainty and improving clinical decision-making. The dual-metric approach enhances the precision of genotype–phenotype correlations and supports the implementation of personalised medicine strategies in disorders associated with Cav3.1 dysfunction.

Clinical implications: The ability to functionally stratify *CACNA1G* variants has direct relevance for clinical practice. Reclassification of VUS can inform genetic counselling, guide therapeutic decisions, and support early intervention strategies. Furthermore, the assay framework aligns with ACMG/ClinGen recommendations, facilitating the incorporation of functional evidence into diagnostic pipelines and variant curation databases.

Functional characterisation of epilepsy associated *KCNT1* mutations: how electrophysiology can help find a cure?

Grigori Rychkov[1,2,3]

[1]Clinical and Health Sciences, University of South Australia, Adelaide, South Australia.
[2]South Australian Health & Medical Research Institute (SAHMRI), Adelaide, South Australia;
[3]School of Medicine, The University of Adelaide, Adelaide, South Australia.

KCNT1 encodes a sodium activated potassium channel, also known as SLACK (**S**equence **L**ike a **C**alcium **A**ctivated **K**$^+$ channel), $K_{Ca}4.1$ or Slo2.2. KCNT1 channel is highly expressed in the nervous system, and is thought to regulate neuronal excitability by modulating hyperpolarization following repetitive firing of action potentials [1]. The strongest evidence of the importance of KCNT1 channels in regulating neuronal excitability came from studies of a *KCNT1* knock out mouse and human phenotypes associated with *KCNT1* mutations. Heterozygous *KCNT1* missense mutations are found in families and individuals with autosomal dominant sleep-related hyper-motor epilepsy (ADSHE) [2], and with the developmental and epileptic encephalopathy (DEE) syndrome known as epilepsy of infancy with migrating focal seizures (EIMFS), which is usually severe, with frequent focal seizures refractory to available epilepsy treatments [3]. *KCNT1*-related epilepsy can be associated with comorbidities including intellectual disability, autism and behavioural features, and can lead to premature death. Majority of known *KCNT1* mutations that cause epilepsy are gain-of-function mutations producing K^+ currents up to several-fold larger than WT KCNT1 [4].

In this work we used *in silico* modelling, patch clamping of HEK293T cells heterologously expressing KCNT1 channel variants, and *Drosophila* model of *KCNT1* epilepsy to investigate the effects of several *KCNT1* mutations on the biophysical properties of KCNT1 currents, to screen a bank of known drugs for potential KCNT1 inhibitors, and to test the efficacy of the identified candidates in blocking KCNT1 currents and preventing seizures in *Drosophila* expressing human KCNT1 channel variants.

All, except one, mutations investigated in this work produced KCNT1 currents of larger amplitude, compared to the WT channel. Some mutations have also affected the kinetics of activation as well as the voltage dependence of the apparent open probability (P_o). Spearman correlation analysis of the characteristics of the KCNT1 channel variants and the severity of the epilepsy caused by the mutations in humans suggested that KCNT1 P_o at the resting membrane potential is a strong predictor of the severity of the disease. *In silico* screening of the Drugbank compound library against the crystal structure of KCNT1 intracellular pore vestibule identified nine molecules that could potentially block KCNT1 channel. Whole cell patch clamping narrowed down the list of the potential candidates to four, two of which, antrafenine and nelfinavir mesylate, blocked KCNT1 current in inside-out patches at nanomolar range of concentrations (IC_{50} ~ 10 – 20 nM). Four drugs identified by the structural modelling and whole cell patch clamping, were selected for *in vivo* analysis to determine their effects on the seizure phenotypes in transgenic *Drosophila* lines expressing KCNT1 channels with patient-specific *KCNT1* mutations G288S, R398Q or R928C. The bang-sensitive behavioral assay ascertained that antrafenine and nelfinavir mesylate added to the food significantly reduced (by 25-50%) seizures in *Drosophila* mutant *KCNT1* lines in a dose dependent manner [5]. Further evaluation of the pharmacodynamics and pharmacokinetic properties of each of the drugs identified here using mammalian models of *KCNT1* epilepsy will be required to justify their clinical evaluation in human patients.

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Functional consequence of pathogenic *GABRA3* variants determines whether X-linked inheritance is dominant or recessive

Philip K. Ahring[1], Katrine M. Johannesen[2], Khaing Phyu Aung[3], Vivian W. Y. Liao[1], Nathan L. Absalom[4], Han Chow Chua[1], Xue Ning Gan[3], Miaomiao Mao[3], Chaseley E. McKenzie[3], Hian Mun Lee[3], Sebastian Ortiz[2], Mary Chebib[1], Guido Rubboli[2], Rikke S. Møller[2], Christopher A. Reid[3].

[1]The Brain and Mind Centre, Faculty of Medicine and Health, The University of Sydney, NSW, Australia. [2]Department of Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre, Filadelfia, Dianalund, Denmark.
[3]The Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC, Australia. [4]School of Science, University of Western Sydney, NSW, Australia.

Background: Disorders of *GABRA3*, the only epilepsy-associated $GABA_A$ receptor subunit gene on the X chromosome, have long eluded clinical clarity due to perplexing observations regarding inheritance patterns and phenotypic variability. The prevailing assumption that all pathogenic variants result in loss-of-function has further oversimplified the landscape, obscuring meaningful genotype-phenotype relationships and stalling progress.

Methods: Here, we curated the largest known cohort of individuals with *GABRA3* variants. By integrating deep phenotyping, genotyping, family history, electrophysiological data, and a targeted mouse model harbouring a recurrent variant, we constructed a coherent picture of *GABRA3*-related disease. All animal experiments were approved by the Animal Ethics Committee at the Florey Institute of Neuroscience and Mental Health.

Results: Forty-three individuals harbouring 19 variants were assembled and our functional assessment reveal that pathogenic *GABRA3* variants exert either gain- or loss-of-function effects, each associated with distinct clinical manifestations. Gain-of-function variants are linked to severe, treatment-resistant epilepsy and severe-profound intellectual disability, disproportionately affecting males, who frequently have hypotonia, non-ambulation, and cortical visual impairment. Conversely, loss-of-function variants yield milder phenotypes, with epilepsy rarely observed. Affected males had behavioural problems and delayed language development, while females were unaffected carriers. Our mouse model with a gain-of-function variant mirrored these sex-specific differences, showing heightened seizure propensity, premature death, and pronounced gain-of-function electrophysiological findings in cortical neurons.

Significance: These insights not only resolve longstanding enigmas surrounding *GABRA3* but also redefine how we interpret X-linked disorders. They highlight that the functional nature of a variant, not its mere presence, dictates whether a condition manifests dominantly or recessively. This has far-reaching implications for genetic counselling, precision medicine, and the broader understanding of X-linked neurodevelopmental disorders.

HDAC4 myonuclear reprogramming underlies denervation, atrophy, and weakness following musculoskeletal trauma

Christopher S Fry

Center for Muscle Biology, University of Kentucky; Lexington, Kentucky, USA, 40536

Joint injury is accompanied by protracted atrophy and weakness that contributes to substantial functional deficits and disability. We aimed to define molecular effectors of lost muscle size and strength following musculoskeletal trauma (injury to the anterior cruciate ligament [ACL]). We recruited twenty-five ACL-injured participants from whom we obtained quadriceps muscle biopsies from ACL-injured and Healthy limbs Pre-, 7-days post-, and 4-months post-reconstruction surgery (Sx) and strength measures up to 6-months post-Sx. We performed immunohistochemistry, RNA-sequencing, HDAC4 chromatin immunoprecipitation sequencing (HDAC4 ChIP-seq), and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) to determine HDAC4 coordination of gene expression changes, in addition to muscle size and muscle strength following injury. We validated HDAC4 as a critical effector of muscle pathology with a mouse model that allowed inducible Hdac4 deletion prior to ACL transection surgery (ACLT; mice underwent surgery under 2.5% isoflurane). Pre-Sx, 49 genes including HDAC4, were differentially expressed between Healthy and Injured limb quadriceps. 7d post-Sx, 5,482 genes were differentially expressed. Integrated HDAC4 ChIP-seq and RNA-seq revealed Pre-Sx HDAC4 binding to and repression of critical muscle genes 7d post-Sx that correspond to gene ontology pathways related to myofibril contraction and aerobic respiration. Repression of these genes coincided with lower muscle fiber cross-sectional area (Healthy: 4845±213.4μm2; pre-Sx: 4313±190.6μm2, $p<0.05$; 7d post-Sx: 3685±228.1μm2, $p<0.05$; 4-months post-Sx: 3422±142.8μm2, $p<0.05$) and peak torque in the Injured limb (Healthy: 174.8±10.72nm; pre-Sx: 135.2±10.19nm, $p<0.05$; 4-months post-Sx: 82.64±6.80nm, $p<0.05$; 6-months post-Sx: 105.9±8.26nm, $p<0.05$) that was sustained throughout follow-up. We then used a $HSA^{merCremer/+}$:$Hdac4^{fl/fl}$ mouse to conditionally delete (Hdac4mKO) or retain (Hdac4-WT) Hdac4 in muscle prior to ACLT. Hdac4mKO mice preserved muscle size and strength at 7d post-ACLT compared to Hdac4-WT mice when presented as a percent change from the non-injured limb (muscle size: Hdac4-WT: -15.20±2.52%, Hdac4mKO: +7.45±12.19%, $p<0.05$; peak torque: Hdac4-WT: -61.79±14.27%, Hdac4mKO: -27.38±24.82%, $p<0.05$). We show that HDAC4 coordinates a repressive gene signature following joint injury by targeting genes critical for muscle function, and muscle-specific deletion of Hdac4 in mice preserved size and strength following injury. Our datasets provide a platform for discovery of genes associated with robust functional deficits in otherwise healthy patients, and we demonstrate the potential of anti-HDAC4 strategies to enhance muscle function and recovery following injury.

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Exploring the strategies of skeletal muscle growth and ageing in fish

Avnika Ruparelia[1,2,3], Yansong Lu[3], Peter Currie[3].

[1] Centre for Muscle Research, Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, 3010, Australia.
[2] Department of Anatomy and Physiology, School of Biomedical Sciences, Faculty of Medicine Dentistry and Health Sciences, University of Melbourne, Melbourne, Victoria, 3010, Australia.
[3] Australian Regenerative Medicine Institute, Monash University, Wellington Road, Clayton, Victoria, 3800, Australia

In vertebrates, growth strategies are amazingly diverse. While mammals and birds are determinate growers that reach their maximum size around sexual maturity with no significant growth thereafter, most teleost species are indeterminate growers that continue to grow with age, without hitting a growth plateau. Evolutionary theories have long predicted a strong correlation between indeterminate growth and negligible ageing but yet the underlying mechanisms remain poorly understood. Using histological and genetic approaches combined with novel image processing workflows, we investigated skeletal muscle growth and ageing in three teleosts with distinct growth capacities: African killifish (*Nothobranchius furzeri*), a determinate grower that undergoes rapid growth followed by rapid ageing; zebrafish (*Danio rerio*), a determinate grower that grows gradually followed by gradual ageing; and giant danio (*Devario malabaricus*), believed to be an indeterminate grower with negligible ageing. Our analyses reveal striking differences in the growth mechanisms used by the different teleost species and identifies molecular mechanisms that could be driving them. Ultimately, our hope it to harness this knowledge to promote muscle growth to sustain muscle health during ageing.

Rethinking the role of growth signals in aging skeletal muscle

Daniel J. Ham, Alexander S. Ham, Marco Thürkauf, Julia Schedel, Ines Böhm, Daniele Reggio, Hirotaka Araki and Markus A Rüegg

Biozentrum, University of Basel, Basel, Switzerland.

As global life expectancy continues to climb, maintaining skeletal muscle function is increasingly essential to preserve life quality for aging populations. We recently identified mTORC1 activation as a hallmark of sarcopenia (Ham et al. 2020), the age-related loss of muscle mass and function. While long-term treatment with the mTORC1 inhibitor rapamycin (RM) predominantly exerted anti-sarcopenic effects in mice, it also induced a pro-aging phenotype and gene expression signature specifically in the gastrocnemius muscle. This signature, which included *Hpgd*, a validated hallmark of sarcopenia (Palla et al. 2021), and *Cdkn1a*, a marker of muscle fibre senescence (Zhang et al. 2022), presented a unique opportunity to further characterise the mechanisms contributing to sarcopenia and identify potential treatment targets. Using single-nucleus (sn) RNA-seq on gastrocnemius muscle from adult (10-month) and old (28-month) control or RM-treated mice, euthanised via sodium pentobarbital (60 mg/kg), we localised the RM-induced, pro-aging signature to a distinct subclass of myonuclei characterised by reduced expression of muscle fibre structural genes and enrichment for diverse aging-related genes. These ‘aging myonuclei’ appeared only in geriatric muscle and were more prevalent in RM-treated mice. Further sub-clustering identified small groups of ‘aging myonuclei’ enriched for genes involved in ‘muscle development’ and ‘denervation’ along with larger groups displaying shared and distinct marker genes for ‘senescence’ and ‘atrophy’. To link pro-aging gene expression to muscle fibre phenotypes, we used highly multiplexed RNA-fluorescence in situ hybridisation (FISH) to visualise the expression of genes from ‘denervation’, ‘senescence’, and ‘atrophy’ clusters in muscle cross sections simultaneously. Furthermore, using this signature, we identified the proto-oncogene c-Myc (hereafter Myc), a potent transcription factor that stimulates ribosome biogenesis, as a potential contributor to sarcopenia. We have recently shown that Myc overexpression rapidly perturbs muscle fibre homeostasis (Ham et al. 2025). Sarcopenic muscle fibres also show elevated *Myc* expression and muscles from 28-month-old HSA-Cre MycKO mice are heavier than those of littermate controls and display lower expression of multiple pro-aging genes. In summary, we leveraged a serendipitous, muscle-specific side effect of rapamycin treatment to identify, localise, and interrogate pro-aging genes in sarcopenic muscle.

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Abstract: SS21

Hepatic SEC16B regulates lipid homeostasis by coordinating VLDL secretion and lipid droplet expansion

Wei Lu[1], Ruicheng Shi[1], Ye Tian[1], Zhiming Zhao[1], Donald Molina[2], Tiantian Yang[1], Xinyuan Zhang[1], Raja Gopoju[3], Yanqiao Zhang[3], Jaume Amengual[2,4], Bo Wang[1,4,5]*

[1]Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Urbana, IL, USA.

[2]Department of Food Science and Human Nutrition, University of Illinois Urbana-Champaign, Urbana, IL, USA.

[3]Department of Internal Medicine, University of Arizona College of Medicine – Phoenix, Phoenix, AZ, USA.

[4]Division of Nutritional Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA.

[5]Cancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana, IL, USA

* Correspondence: bowang@illinois.edu

The liver plays a critical role in lipid homeostasis, where lipids are either secreted as very-low-density lipoproteins (VLDL) or stored in lipid droplets (LDs). However, the regulatory mechanisms governing these two interconnected processes remain poorly understood. Here, we demonstrate that SEC16B is crucial for regulating lipid secretion and storage within the liver. Genome-wide association studies have identified single-nucleotide polymorphisms in *SEC16B* to be highly associated with serum lipid levels in humans. Hepatic *Sec16b* deficiency decreases serum lipid levels due to impaired MTP-independent VLDL lipidation and COPII-mediated intracellular trafficking. SEC16B partially localizes at ER-LD contact sites and promotes LD expansion by facilitating the targeting of ER proteins to LDs. More importantly, suppression of *Sec16b* dramatically lowers serum lipid levels and reduces atherosclerotic lesion size in *Ldlr* null mice. These data reveal a novel mechanism that coordinates VLDL and LD metabolism and suggest SEC16B as a promising therapeutic target for atherosclerosis treatment.

Abstract: SS22

The Brain Is Activin Up

Cait A Beddows[1], Paul Gregorevic[1,2], & Garron T Dodd[1]

[1]Department of Anatomy & Physiology, The University of Melbourne.
[2]Centre for Muscle Research, The University of Melbourne.

The clinical development of the Activin receptor antagonists, such as Bimagrumab, highlights Activin signalling as a promising and emerging pharmacological target for metabolic disease. Although the roles of Activin signalling in peripheral tissues, such as muscle and adipose tissue, are relatively well characterised, its functions within the central nervous system (CNS) remain poorly understood. Bridging this gap may reveal new therapeutic avenues beyond those already explored within peripheral systems.

We identify Activin signalling as a key inflammatory pathway activated in the mediobasal hypothalamus (MBH) during obesity. Using an AAV-mediated approach delivered stereotaxically within the ARC under isoflurane anaesthesia (1–2% inhalation, oxygen-enriched), we demonstrate that mice exposed to a high-fat diet are protected from weight gain, glucose intolerance, and increased food intake when endogenous Activin signalling in the MBH is attenuated. Conversely, promoting Activin signalling in the MBH of lean mice using AAV overexpression drives metabolic dysfunction, including elevated body weight, adiposity, food intake, and impaired glycaemic control. These findings establish hypothalamic Activin inflammation as a critical driver of metabolic disease.

The specific CNS cell types mediating Activin's role in metabolism remain largely unexplored. Here, we demonstrate that enhanced Activin signalling within the MBH drives proliferation and reactive changes in NG2 glial cells, a cell population increasingly recognised for its contributions to CNS plasticity and disease. To selectively ablate proliferating NG2 cells, we employed an AAV-HSV-thymidine kinase strategy delivered stereotaxically under isoflurane anaesthesia, followed by intraperitoneal ganciclovir administration (25 mg/kg every 3 days). Preventing NG2 cell reactivity, either during high-fat diet exposure or in the context of AAV-induced hypothalamic Activin inflammation, protected against metabolic dysfunction. All tissues were collected from animals under terminal anaesthesia or following humane euthanasia in accordance with approved animal welfare protocols. Mice lacking reactive NG2 cells preserved normal glycaemic control and exhibited reduced food intake and weight gain, even under conditions of heightened Activin signalling in the MBH.

Together, these results provide the first causal evidence that hypothalamic Activin signalling contributes to metabolic disease and identify NG2 cell reactivity as a key downstream effector. This work connects a clinically validated pathway with previously unrecognised CNS mechanisms, highlighting central Activin signalling as a potential therapeutic axis that could compliment peripheral strategies such as Bimagrumab.

The Guts of the Matter: Intestinal Ceramides are Metabolic Gamechangers

Michael Mah[1], Nikolas Scott[1], Enyuan Cao[1], Dovile Anderson[1], Colby Zaph[2], Natalie Trevaskis[1], Mark A Febbraio[1], Sarah Turpin-Nolan[1]

[1]Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia
[2]Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Ceramides, a class of bioactive sphingolipids, are critical regulators of metabolic health. While the liver has long been regarded as the primary site of ceramide synthesis and export, we[1] and others have identified the small intestine as an overlooked yet important contributor to systemic ceramide homeostasis. Dietary lipid intake strongly modulates intestinal ceramide production, with high-fat feeding driving increased generation and export of C16:0 ceramides packaged in chylomicrons. Elevated C16:0 ceramides are closely associated with obesity, insulin resistance, and hepatic steatosis, whereas very long-chain species such as C22:0 and C24:0, produced by ceramide synthase 2 (CerS2), are linked to improved metabolic outcomes.

Abstract: P1

Establishment of a Human iPSC Model of the Cav1.2 L1521I Variant: Insights into Cardiac Differentiation and Characterization

Yuan-Chin Lee[1], Evan Ingley[2,3] and Livia Hool[1,4]

[1]Ben Beale Laboratory in Cardiovascular Research, School of Human Sciences, The University of Western Australia, Perth, WA, Australia.
[2]Harry Perkins Institute of Medical Research, Nedlands, WA, Australia.
[3]Murdoch University, Murdoch, WA, Australia.
[4]Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia.

Background: The voltage-gated calcium channel Cav1.2 is the predominant type found in heart muscle. Several missense variants in the α1c subunit of Cav1.2 are associated with abnormal function, including long QT syndrome phenotypes, as reported in the ClinVar database.

Objective: To generate human induced pluripotent stem cells (hiPSCs) carrying the Cav1.2 L1521I mutation, differentiate them into cardiomyocytes (CMs), and characterize their molecular and cellular properties.

Methods: The L1521I point mutation was introduced into hiPSCs using CRISPR-Cas9 gene editing with single-stranded donor DNA (ssODN) homology directed repair (HDR). hiPSCs transfected with Cas9/sgRNA, but without ssODN served as no-donor (HDR-negative) controls (hiPSC-Cas9C). Genome editing was validated by Sanger sequencing. Pluripotency was assessed by flow cytometry and immunocytochemistry (ICC) for SOX2 and SSEA4. Cardiomyocyte differentiation was induced through temporal modulation of WNT/β-catenin signaling. Cardiac identity was confirmed by ICC and flow cytometry for cardiac troponin T (cTnT), and subtype composition (ventricular vs. atrial) was assessed by marker (MYL7, MYL3) expression.

Results: Sanger sequencing confirmed successful introduction of the Cav1.2 L1521I variant with no detectable indels in hiPSC-L1521I lines. Both hiPSC-L1521I and hiPSC-Cas9C retained high pluripotency marker expression (>90%) and displayed normal morphology. Directed differentiation yielded functional cardiomyocytes with >85% cTnT-positive cells, as confirmed by ICC and flow cytometry. Derived cardiomyocytes exhibited mixed ventricular and atrial marker expression. Importantly, the presence of the L1521I mutation did not affect pluripotency maintenance or cardiomyocyte differentiation efficiency.

Conclusion: We have established a genome-edited hiPSC model carrying the Cav1.2 L1521I mutation that differentiates efficiently into cardiomyocytes, providing a platform to study the cardiac electrophysiology of Cav1.2 variants in biological relevant cells.

Effects of fecal microbiota transplantation from exercised and/or inulin-supplemented donors on physical and metabolic parameters in diet-induced obese mice

Masato Kawashima[1,2], Takafumi Aoki[3], Hiroki Hamada[2,4], Chihiro Watanabe[2], Eri Oyanagi[2], Takumi Kono[4], Takashi Yamagata[2], Hiromi Yano[2,4].

[1]Department of Humanities and Social Sciences, Institute of Liberal Arts, Kyushu Institute of Technology, Kitakyusyu, Japan, [2]Kawasaki University of Medical Welfare, Department of Health and Sports science, Kurashiki, Japan, [3]Kawasaki University of Medical Welfare, Department of Clinical nutrition, Kurashiki, Japan, [4]Kawasaki University of Medical Welfare, Graduate school of Health Science and Technology, Kurashiki, Japan.

Fecal microbiota transplantation (FMT) has emerged as a potential therapeutic approach for obesity and associated metabolic complications. Both habitual exercise and dietary fiber intake, such as inulin supplementation, are known to induce distinct alterations in the gut microbiota and to exert additive or synergistic benefits on metabolic health. In this study, we sought to determine whether transferring gut microbiota from lean donor mice exposed to regular exercise and/or inulin supplementation could mitigate metabolic impairments in mice with high-fat high-sugar diet (HFHSD)-induced obesity. Four-week-old male C57BL/6J mice were assigned to donor (n = 24) and recipient (n = 60) groups. Recipients were maintained on HFHSD and allocated to one of five treatments: sham FMT (Sham), FMT from sedentary donors (Sed-R), exercised donors (Ex-R), inulin-supplemented donors (Sed + Inu-R), or donors receiving both interventions (Ex + Inu-R). Following 12 weeks of obesity induction, these recipient mice were treated with antibiotics (1 mg/ml ampicillin and 0.5 mg/ml neomycin in drinking water) and then underwent a 4-week FMT protocol. During the FMT period, crude aqueous fecal extracts from donors were administered to the respective recipient mice via oral gavage (100 µl each). Glucose tolerance tests, involving intraperitoneal glucose injection (2 g/kg body weight), were conducted before and after FMT to assess glucose homeostasis. At the end of the feeding period, the mice were euthanized by cervical dislocation and tissue samples were collected. Physical and metabolic parameters, gut microbiota profiles, and cecal short-chain fatty acid (SCFA) levels were evaluated in both donors and recipients. The results showed that none of the FMT-treated groups (Sed-R, Ex-R, Sed + Inu-R, and Ex + Inu-R) exhibited improvements in obesity-related features, such as body weight or relative fat mass, compared with the sham group. Similarly, FMT interventions failed to ameliorate circulating metabolic indices or glucose intolerance. These outcomes were accompanied by only partial alterations in gut microbiota and SCFA profiles. These findings suggest that prolonged HFHSD feeding hinders the engraftment and function of beneficial microbes, thereby attenuating the metabolic benefits of FMT.