Scientia

SHAPING THE FUTURE OF HEALTH AND HEALTHCARE

HIGHLIGHTS:

- The Voluntary Stopping of Eating and Drinking: Dying with Dignity?
- Determining the Link Between Diet and Cancer
- Targeting the Immune System to Our Advantage
- Repairing the Cornea: A New View on Novel Therapies

EXCLUSIVE:

- Antibiotic Research UK

Issue: #139

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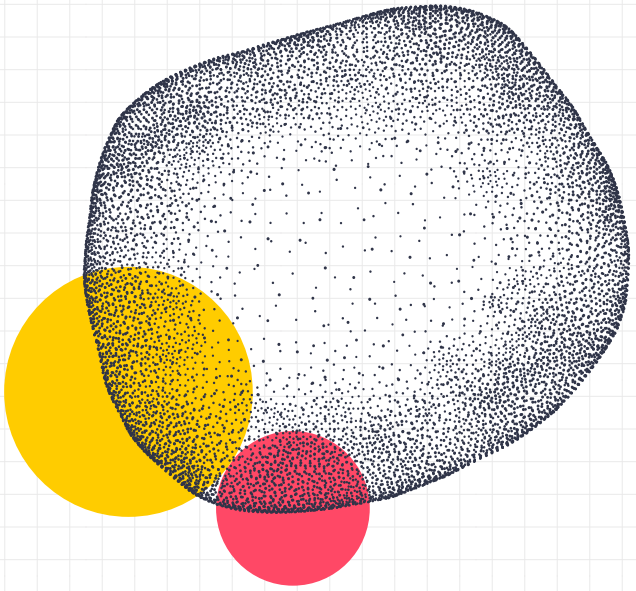

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WELCOME...

This important issue of Scientia takes an exciting look into the future of health and healthcare delivery across the world. For more than a year, COVID-19 has dominated almost every aspect of our lives, causing unprecedented disruption on a global level. For this reason, it is with great pride that we celebrate the significant advances in science, health and healthcare that have been achieved in the face of such adversity.

Our first section showcases the dedicated efforts of researchers working to improve the promotion of healthy living as well as the provision of clinical care and disease prevention through policy development. From understanding and ensuring the provision of safe drinking water to guiding the development of nursing and midwifery in the community, we read of a wide range of evidence-based approaches that are transforming healthcare policy and practice.

The second section focuses on the researchers tackling the causes and consequences of poor nutrition. We read how the predominantly developing world problem of undernutrition has received the attention of a collaboration of experts who are challenging traditional assumptions and providing a vital evidence base to inform future practice in this field. On the opposite end of the spectrum, we read of the researchers working to overcome the causes and consequences of obesity, including the causal impact of mass media on overeating in young adults and the potentially fatal onset of pancreatic disease.

Our third section highlights the work of researchers dedicated to progressing understanding of how we fight infection and defend against disease. In an exclusive interview, we meet Professor Colin Garner, founder and Chief Executive at Antibiotic Research UK, and gain insight into their work supporting research into new antibiotic treatments, educating the public about the dangers of drug-resistant infections and providing the UK's first patient support service. From improving the diagnosis of potentially fatal responses to infection to marvelling at the surprising benefits that bacteria can bring, we are reminded of the critical importance of this field in healthcare.

Our fourth section celebrates the work of scientists who are dedicated to the development of innovative treatments and technologies in health and healthcare. In this riveting final section, we read of pioneering approaches to reducing the incidence of preterm birth, novel therapies to regenerate damaged heart muscle and how insights from music are being used to improve the quality of the auditory signals of life-saving devices used in hospitals across the world.

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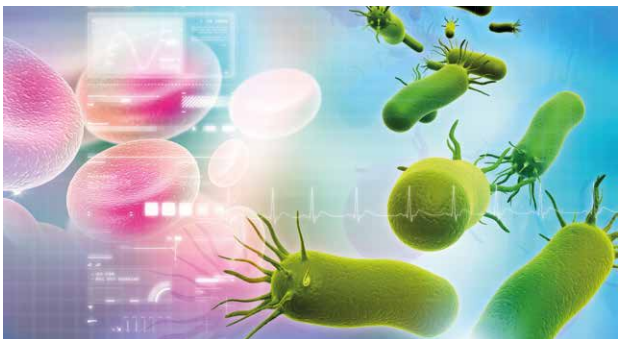
Meet The Team...

CONTENTS

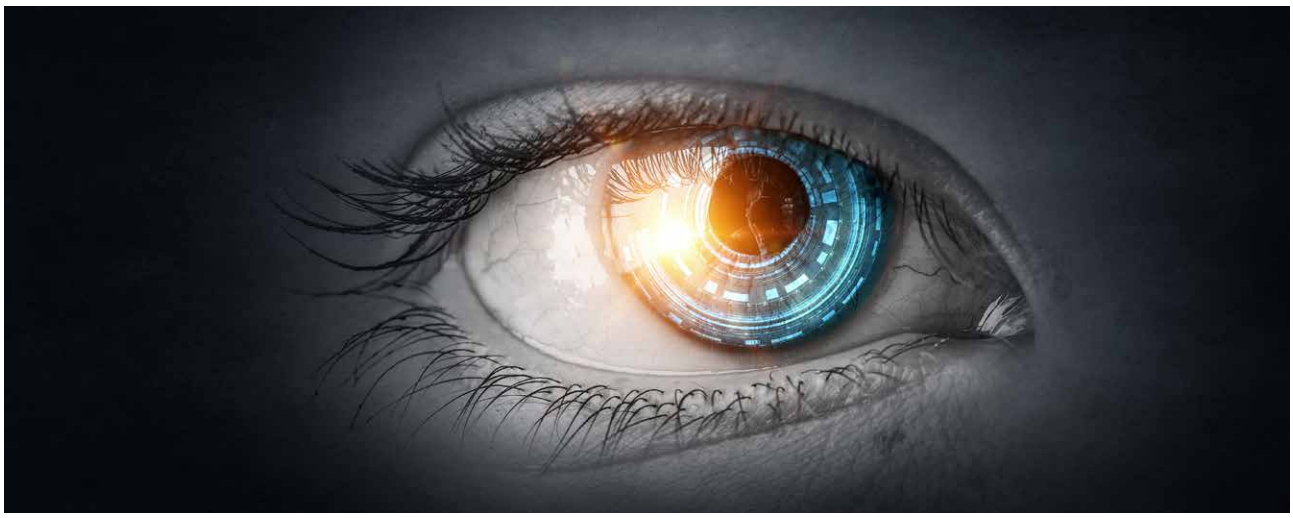
ISSUE : #139

POLICY AND PRACTICE

DISORDERS OF NUTRITION



INFECTION AND THE IMMUNE SYSTEM



EMERGING TREATMENTS AND TECHNOLOGIES

POLICY AND PRACTICE

IMPROVING HEALTH AND WELL-BEING: FROM POLICY TO PRACTICE

The first section in this issue is dedicated to the researchers working to bring about important improvements to health and well-being by informing and advancing health policies with vital research evidence. Health policy, as defined by the World Health Organization, refers to the 'decisions, plans, and actions that are undertaken to achieve specific health care goals.' Health policies are critical for the promotion of healthy living, provision of clinical care and disease prevention, and as we see in this section, can vary drastically across local, national, regional and global levels.

We open this section by meeting Professor Patricia Leahy-Warren at the Catherine McAuley School of Nursing and Midwifery at University College Cork. Professor Leahy-Warren leads a collaboration of experts guiding the development of nursing and midwifery in the community in Ireland. Primary care is increasingly being embraced as central to healthcare services, and we read of the importance of Professor Leahy-Warren's work in filling the complex but critical gaps in the existing literature required to inform best practice.

Professor Dr André Fringer and Mrs Sabrina Stängle at the Zurich University of Applied Sciences also take a nursing perspective in the improvement of healthcare. Their focus is on the controversial but poorly understood means to hasten death through the voluntary stopping of eating and drinking as an alternative to active euthanasia. We read how their work is driving forward our understanding of the medical and ethical ramifications of this practice with the potential to improve end-of-life care in Switzerland and further afield.

Dr Natalia Sira from East Carolina University is improving patient care by taking a holistic and individualised approach to health outcomes, treatment and rehabilitation. We read how connecting body and mind through the consideration of both the physical and psychological components of health helps determine our reactions and behaviour. Dr Sira argues that the ways in which we achieve our optimal potential determine how well we adapt and cope with changes in our environment, deal with stresses in life and maintain overall well-being.

Around the world, the depletion of resources accompanied by rising demands puts healthcare systems under increasing pressure. Dr Johannes Bircher provides important reflections on the inherent failings of modern healthcare systems, culminating in his proposal for a new concept of health – the Meikirch model. We read how this new framework for understanding health has the potential to drive forward a critical paradigm shift in healthcare delivery.

One specific depletion of resources in healthcare systems is the (un)availability of healthcare professionals, and this negatively impacts the quality of care that can feasibly be delivered. Dr Sarah Hewko, based at the University of Prince Edward Island, is conducting valuable research into the reasons why health professionals (and nurses in particular) retire earlier than planned. We read how her work is providing knowledge that will be key to building and maintaining sustainable healthcare systems in Canada and further afield.

We then turn to the work of Dr Steve E. Hrudey from the University of Alberta. The provision of clean, safe drinking water is a basic human need, and the contamination of drinking water can lead to disease and death. Dr Hrudey is working to understand and ensure the provision of safe drinking water and has outlined critical recommendations to protect public health. We also read of the challenges to providing safe drinking water in developed countries and the common misconceptions surrounding this.

Fracking has long been controversial for its potential to contaminate local water sources and cause damage to the environment. Dr Kathleen Mullen at Littleton Equine Medical Center in Colorado and her team are researching how fracking in close proximity to a horse-breeding farm may be the cause of a specific birth defect in the foals born there. We read how her important findings may be relevant to human babies and considerations of the implications of fracking on long-term health.

Professor Mary Rezk-Hanna from the University of California works with a group of scientists aiming to drive the policy regulation of tobacco and alternative tobacco products. Hookah smoking is the least regulated form of tobacco but is rapidly gaining in popularity, particularly among youth and young adults. We read of Professor Rezk-Hanna's critical work investigating the effects of alternative tobacco products on the cardiovascular system and the implications of this on health.

Although the potential health risks of tattooing are well-established, many of the regulations that govern the practice of tattooing are somewhat relaxed compared to other industries. Dr Christopher Hohl and the Chromatography Section at the State Laboratory Basel-City, Switzerland, work to analyse the composition of tattoo inks and investigate the effects of tattooing. We read how Dr Hohl's work is providing the relevant authorities with the key evidence they require to improve tattoo safety standards.

We conclude this section by meeting Dr Logan M. Lawrence from Dalhousie University, Nova Scotia. Dr Lawrence explores the concept of 'policy capacity' to try and understand, measure and operationalise the best approaches to assist and support policy makers to make 'the right decisions'. We read how his approach of evidence-informed decision making is breaking down the walls between health researchers and those responsible for developing policies.

EXPLORING MODELS OF NURSING AND MIDWIFERY IN THE COMMUNITY: INTERNATIONAL AND NATIONAL PERSPECTIVES

Across the world, primary care is increasingly being embraced as central to healthcare services. Effective delivery requires an efficient and cost-effective community-based model of care. However, no single overarching model of nursing and midwifery practice in the community exists in the literature. A collaboration of experts, headed by **Professor Patricia Leahy-Warren** from the Catherine McAuley School of Nursing and Midwifery at University College Cork in Ireland, is addressing this complex but critical gap to guide the development of nursing and midwifery in the community in Ireland.

Nursing and Midwifery in the Community

Internationally, primary healthcare is accessed within the community, usually at a GP practice or one's own home. For many individuals, this is where they first come into contact with healthcare professionals and also where they receive care. In addition to providing treatment, increasing emphasis is also being placed on the promotion of health and well-being, and the prevention of disease within the community. Nurses and midwives are at the forefront of this fundamental provision, constituting the largest group of professionals working in the community context.

The diversity in nursing in the community is reflected in the roles and titles assigned to registered nurses – these can include health visitor, public health nurse, school nurse, practice nurse, clinical specialist nurse, advanced nurse practitioner and generalist nurse. Furthermore, the role of the nurse or midwife varies across individual and population groups. It involves differing emphasis on preventative care, health education, health promotion, case management, and self-management support.

To add to this complexity, the provision of nursing and midwifery in primary healthcare is confronted by a multitude of challenges. Populations and communities are multifarious in their approaches, resources and needs. Globally, ageing populations and chronic ill-health rates are increasing, accompanied by ongoing developments in health, social and welfare provision, all pointing to the need for proactive (rather than reactive) multifaceted service healthcare provision in the community.

The Need for Re-orientation

Given the inherent complexity in existing services and the current challenges these services face, it is argued internationally that healthcare provision, in its current form, requires dramatic change or 're-orientation' in the coming years. More specifically, it is

argued that this re-orientation should ensure more effective and efficient provision of services, underpinned by improved partnerships and systems, stronger leadership and governance, and a greater focus on evaluation. In recognition of this, the Department of Health (DoH) in Ireland commissioned a review of evidence on current models of nursing and midwifery practice in the community, with the aim of informing policy development.

The DoH awarded the tender to a consortium of universities (University College Cork, National University of Ireland, Galway, and University College Dublin), led by Professor Patricia Leahy-Warren from the Catherine McAuley School of Nursing and Midwifery, University College Cork.

Reviewing the Literature on Nursing and Midwifery in the Community

Professor Leahy-Warren and her colleagues undertook a rigorous systematic review of empirical and grey literature on nursing and midwifery in the community. They identified 127 empirical and 24 grey literature papers based on searches conducted on multiple databases, which they evaluated and analysed to identify broad categorical themes and associated subthemes.

It is of note, that despite the breadth of literature explored by the team, that not one single overarching model of nursing and midwifery practice in the community was identified. Importantly, however, the team uncovered valuable insights that they synthesised into four core themes to inform the development of a proposed model to overcome this gap in the literature.

Integrated and Collaborative Care

The first theme identified focuses on the need for greater linkage between primary services (day to day healthcare and the first port of call for health advice and treatment) and secondary services as broadly defined, to ensure all sectors are working in tandem. They argue that this is crucial for maintaining the overall well-being of a population, as it represents the link between all sectors of the health service, including nurses, midwives, and specialised practitioners.

The successful integration of all aspects of care would benefit patients with complex needs (such as those with multiple and ongoing conditions) in particular. Professor Leahy-Warren and the team noted that nurse-led care for chronic diseases such as diabetes and continence management compares favourably to physician-led care, and that midwife-led care for low-risk pregnancies is generally cost-effective, safe and well-received by women.

Organisation and Delivery of Nursing and Midwifery Care

The second theme is focused around the organisation and delivery of nursing and midwifery care in the community. Translational care models, such as home-based, telehealth (which utilises electronic information and telecommunication technologies to provide health services), and nurse-led care are associated with positive outcomes for patients as well as higher satisfaction. This kind of approach is especially important for vulnerable or marginalised populations, and Professor Leahy-Warren and her colleagues emphasise the importance of these interventions for a strong primary healthcare system. In support of this, they cite high quality studies demonstrating the use of home-based and nurse-led interventions as having a strongly positive influence and being more cost-effective than usual medical care.

Adjuncts to Nursing and Midwifery

The third theme identified by Professor Leahy-Warren and her colleagues relates to adjuncts to nursing and midwifery in the community. Adjuncts to nursing, such as the support of non-licenced personnel working under the supervision of a nurse, have been shown to have significant positive effects primarily on maternal and child health and emotional well-being. The approach of helping patients help themselves is a hugely important aspect of healthcare, and nurse-led interventions with supporting personnel can help reduce the risk of hospitalisation in young adults with chronic disease.

Within this theme, the importance of embracing technological developments also becomes clear. It has been demonstrated that the delivery of healthcare remotely using telehealth interventions is associated with clinical benefits, including providing support for patients to feel empowered to participate in their own care. This type of approach could be combined with chronic care plans, such as for patients with cystic fibrosis, providing an access point for care almost anywhere and in particular, providing a significant benefit for rural communities.

In the current day and age, and particularly during the current COVID-19 pandemic, adjuncts to nursing are likely to become increasingly important in modernising the ways in which we access healthcare although, so far, their effectiveness has only been evaluated to a limited extent.

A Proposed Model of Nursing and Midwifery in the Community

Professor Leahy-Warren and her colleagues' extensive review identified key evidence for the core components of community and nursing and midwifery that should underpin any future interventions. These core components of nursing and midwifery are presented within a framework informing how a model might be conceptualised. Through this approach, it can become possible to optimise the full potential of the implementation of evidence-based nurse-led and midwifery-led care.

Of particular importance within this framework is the adoption of a care management, person-centred approach in which patients actively participate in their own care, working in close cooperation with healthcare professionals. Acknowledging the importance of provisions such as home-based interventions and telehealth support is essential if we want to improve the way we care for our communities. However, further research on such interventions is required.

Prior to the work of Professor Leahy-Warren and her colleagues, there had been little attempt to understand the complexity of nursing and midwifery services in the community. Each patients' lifetime, from birth to death, encompasses a huge range of services, professionals, health conditions and events which would benefit from a strong and integrated approach. Indeed, taking the person-focused perspective turns healthcare into a much more compassionate system which may have more positive outcomes down the line, reducing the burden on healthcare services.

Improving the Lives of People in Ireland and Beyond

The foundations of good care, based on the philosophy of integration and collaboration as outlined by Professor Leahy-Warren and her colleagues now provide the building blocks for a more fluid and responsive nursing and midwifery service in the community, and one that could seamlessly meet the ever-evolving needs of a population. This solid, theoretically and empirically derived basis for nursing and midwifery in the community is critical if we are to balance policy goals with the practical ability to achieve such goals. To sum up, the ambitious and far-reaching work conducted by Professor Leahy-Warren and her team will inform policy development relevant to nursing and midwifery in the community nationally within Ireland and is likely to be of significant interest to policy makers, researchers and practice leaders on an international scale.

Meet the researcher

Professor Patricia Leahy-Warren
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Cork, Ireland

Professor Patricia Leahy-Warren is *Chair of Academic Council Graduate Studies Committee, Director of Graduate Studies and Chair of the Maternity, Families and Primary Care Research* Group at Catherine McAuley School of Nursing and Midwifery at UCC. She is a registered nurse, midwife, and public health nurse, and an honours graduate from the School of Nursing and Midwifery at UCC. She holds postgraduate degrees in Public Health Nursing, Masters and a PhD in the area of Maternal and Infant health. Her current and past research projects are focused on the perinatal health of women, their infants and partners with a particular interest in *Social Support, Self-Efficacy, Community, Postnatal Depression and Breastfeeding*. She is the recipient of two Clinical Research Fellowships from the HRB. She has secured competitive research funding of €1,408,415 as a Principal Investigator, co-applicant and/or collaborator.

She is currently the Principal Investigator on an HRB Research Project which is Practice Enhancement for Exclusive Breastfeeding (PEEB) across the pregnant woman's Perinatal journey from first confirmation of pregnancy with a GP practice, through the maternity services for antenatal, intranatal and postnatal care and seamless transition to the community and will include public health nurses, GPs and practice nurse services, maternity services and breastfeeding mothers. This project will have a direct impact on decision-making and practices in the knowledge user organisation and make a significant impact on the health and well-being of women, their infants and families.

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Professor Leahy-Warren has published in nursing, midwifery, medical and allied health journals with significant impact factors including six book chapters; 13 commissioned reports; over 80 peer-reviewed publications; 73 peer-reviewed conference papers and 13 invited speaker conference presentations. She has supervised eight research students (Doctoral, Research Masters) to completion and acted as external examiner for six PhD theses in the UK, Northern Ireland, Australia, and Canada.

Professor Leahy-Warren is adjunct Senior Lecturer in the School of Nursing and Midwifery, Western Sydney University, Australia and Visiting Professor at the VID Specialised University, Oslo, Norway. Her philosophy is that all women, infants and their families should be facilitated to have a positive pregnancy and childbirth experience, receiving evidence-based care from all healthcare professionals. Improving maternal health during the perinatal period directly influences and enhances infant outcomes.

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FURTHER READING

P Leahy-Warren, H Mulcahy, L Benefield, C Bradley, A Coffey, A Donohoe, S Fitzgerald, T Frawley, E Healy, M Healy, M Kelly, B McCarthy, K McLoughlin, C Meagher, R O'Connell, A O'Mahony, G Paul, A Phelan, D Stokes, J Walsh, E Savage, Conceptualising a model to guide nursing and midwifery in the community guided by an evidence review, BMC Nursing, 2017(16), 35.


















Left to right:

Professor Patricia Leahy-Warren (PI)[1], Dr Helen Mulcahy (Co-PI)[1], Dr Kathleen McLoughlin[1], Dr Marcella Kelly[2], Professor Amanda Phelan[3], Professor Eileen Savage[1], Professor Lazelle Benefield[4], Professor Colin Bradley[5], Professor Alice Coffey[6], Dr Ann Donohoe[7], Dr Serena Fitzgerald[1], Dr Tim Frawley[3], Dr Maria Healy[8], Bernard McCarthy[2], Catherine Meagher[2], Dr Rhona O'Connell[1], Dr Gillian Paul[3], Diarmuid Stokes[9], Ms Elizabeth Healy[10]

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THE VOLUNTARY STOPPING OF EATING AND DRINKING: DYING WITH DIGNITY?

To die with dignity is a common wish but not one that is easily granted. **Professor Dr André Fringer** and **Mrs Sabrina Stängle**, both of the Zurich University of Applied Sciences, are investigating the voluntary stopping of eating and drinking (VSED) as a means to hasten death as an alternative to active euthanasia. Their work is driving forward our understanding of the medical and ethical ramifications of this practice.

A Critical but Poorly Understood Issue

Removing the ambiguity surrounding euthanasia and assisted suicide could increase the quality of care received by those involved. For many chronically ill people, the desire to die is met with an onslaught of social, legal, and ethical consequences that may beg the question: isn't there an easier way?

For the strong-willed patient, voluntarily abstaining from food and can lead to death by dehydration in anything from days to weeks. The time taken for death to occur depends on a multitude of factors including the health, age, and mental strength of the person involved. Whether this occurs explicitly and as a spoken wish by the patient, or implicitly, as a result of poor health or otherwise, the voluntary stopping of eating and drinking (VSED) is a significant cause of death that has not been extensively recognised in the medical community.

Professor Dr André Fringer and Mrs Sabrina Stängle, both of the Zurich University of Applied Sciences, intend to change this. They have conducted research on VSED for the last several years and brought the debate on whether VSED should be treated as suicide or natural death to Switzerland.

Suicide or Natural Death?

The issue of VSED, also known as 'death fasting', requires professional clarification to achieve uniform support across medical settings. Professor Fringer and Mrs Stängle explain that suicide, described by the World Health Organisation as 'the act of deliberately killing oneself,' may not be applicable to VSED if the individual already has a life-threatening illness. In this case, VSED could be simply thought of as speeding up the inevitable.

However, if an otherwise healthy person decides to undergo this process, it can be more accurately labelled as suicide since premature death ensues. This clarification becomes important when seeking the support of families or medical teams witnessing the dying process. The third position considers patient autonomy. As such, it may be considered unlawful or coercive to force food ingestion upon an unwilling, mentally well patient.

The legality and associated consequences of choosing to die in this manner vary from country to country. In Switzerland, considered a 'right to die' society, subjects who choose VSED are generally viewed as having experienced

a natural death. In Germany and Austria, however, opinions are more divided and discussion around the subject is more controversial. Globally, the phenomenon of VSED exists, though is often hidden due to a lack of open discussion or integration into healthcare systems. For families or medical teams asked to support this request, it comes down to personal choice and the level of perceived suffering at hand.

Ultimately, and despite the surface-level autonomy and independence required, those choosing the VSED path require a palliative care model towards the end of their life. The ensuing vulnerability and loss of functionality that is experienced must be a key factor to be considered by interested parties. Professor Fringer and Mrs Stängle explain that the starting point of the individual's health is what determines whether VSED leads to a natural or radical death.

Oral Nutrition Refusal Sub-types

Due to insufficient material existing on the VSED, Professor Fringer and Mrs Stängle aimed to identify and distinguish between different forms of oral nutrition refusal and different forms of VSED. Following media attention gathered during their interactions with healthcare professionals, the researchers received requests from people who had supported relatives through the VSED who wanted to share their experiences. Open-ended, semi-structured interviews were then conducted with these family members, who were asked to describe the full course of the VSED, including reasons for implementation and the decision-making process involved. Any complications which arose were also explored.

This process revealed that there is much more to the VSED than a simple decision to refrain from eating or drinking. The authors identified that nutrition refusal in VSED should be identified as separate from mental illness, eating disorders, natural death and hunger strike. VSED was also found to exist in implicit forms, driven by the various motives the individual wishing to die may have for refraining from fluids in secret and undetected by others. Thus, although VSED can often be a very explicit form of death involving spoken intent, the research by Professor Fringer and Mrs Stängle demonstrates that much more difficult to detect forms of food and liquid refusal also require better understanding.

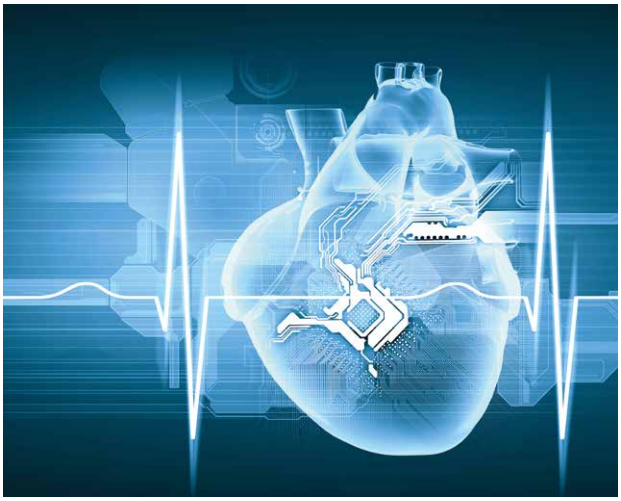
During implicit VSED, the healthcare workers involved may misjudge the process and view it as a natural death. In this example, individuals often feel socially or emotionally isolated and may choose to refrain from food and liquids without explicitly expressing this decision. These patients either do not want to or cannot communicate their decision. This decision can be thought of in the context of 'life fatigue' as opposed to the desire for autonomy. The researchers saw this commonly in old age.

Overall, the researchers clearly showed that VSED is a multifaceted condition that must be distinguished from other forms of nutrition refusal. In particular, implicit and concealed VSED should become known as a form of nutrition refusal in order to avoid confusion with the natural dying process. Different forms of nutrition refusal close to VSED are associated with different needs, and professional support must be adapted accordingly.

A Palliative Care View

Professor Fringer and Mrs Stängle's next step was to ascertain a comprehensive representative picture of VSED in Switzerland, on which no prior empirical data existed. Professor Fringer and Mrs Stängle created and distributed a trilingual survey (in German, French, and Italian) to Swiss healthcare personnel, the results of which would provide important information both about the occurrence of VSED, and also about its professional handling. The aim of this work was to help improve the quality of care received by patients opting for VSED, and to improve competence and confidence in associated healthcare staff.

Additionally, the team's questionnaire aimed to gather details of healthcare workers' attitudes towards VSED, including their willingness to participate in the practice. The survey was distributed to a variety of healthcare professionals, from GPs to nurses to paramedics, since interdisciplinary cooperation is essential for potential future standardisation of care.



Data analysis revealed that the VSED in these settings is mainly carried out by cancer patients (40.5%) or by people without any serious disease (28.9%). The underlying causes include fatigue (61%) and the fear of being dependent on others (59.5%). Death from the VSED was viewed as dignified by 57.5% of all professionals surveyed.

Looking to Nursing Homes

The team also created a cross-sectional study directed at heads of Swiss nursing homes to gather their experiences and opinions on VSED. Their online survey was answered by 34% of all nursing home heads, and data analysis revealed 1.7% of all patients in these settings died following VSED. Almost 70% of participants considered this phenomenon highly relevant in their daily work. Participants described the VSED as being more expected by older patients, and they tended to view it as a natural death which was accompanied by health professionals. As many as 92% of participants believed that patients undergoing VSED deserved the right to care, and overall, views toward the subject were positive.

In general, the survey found that healthcare professionals view VSED as a phenomenon of old age, rather than a formalised end-of-life practice. Professor Fringer and Mrs Stängle's insight indicated the need for additional staff training and clearer positioning on this issue, which would provide essential clarity and better standardisation of care.

Family-centred Care

Alongside creating professional acceptance and integration of the VSED, Professor Fringer and Mrs Stängle explained the need for a family-centred care model to be established to help all those involved. Patients who choose the VSED will at some point depend on familial support, as well as nursing or medical aid. Whether individuals opt for the accompaniment of their relatives during their VSED process, or for the family to assist only with medical or legal formalities, some interaction is necessary between the patient, their family, and medical staff. To this end, Professor Fringer and Mrs Stängle developed a family-centred care model, which would take all of this into account and could improve the wellbeing of all involved in this process.

Professional Stance of Physicians

In recently published research, Professor Fringer and Mrs Stängle aimed to determine how often patients undergoing the VSED do so with the support and/or knowledge of their Swiss family physicians. In addition to this, they were interested in physicians' attitudes and professional stances towards this issue.

After analysing results from 751 practising family physicians, the researchers determined that VSED was a well-known phenomenon among 81.9% of those investigated, with over one-third having accompanied at least one patient during VSED. Despite this, there is still a lack of in-depth knowledge on the subject required to appropriately advise patients and families concerned. In 2017, VSED accounted for 1.1% of all deaths that occurred in Swiss nursing homes or privately. The physicians classified this as a natural dying process (59.3%), passive euthanasia (32.0%), or suicide (5.3%).

Another recent paper by Professor Fringer and Mrs Stängle explored the experiences, personal attitudes, and professional stances of 1,681 Swiss health care professionals (including nursing directors, institute directors and head nurses) toward VSED using a standardised questionnaire. Findings indicated predominantly positive personal attitudes of professionals when confronted with VSED and desire to support the autonomy and self-determination of patients despite potentially holding moral reservations.

It is clear that further training and the development of basic practice recommendations relating to VSED is required to better support both healthcare personnel and patients. The awareness raised by Professor Fringer and Mrs Stängle is critical in paving the way for improved end-of-life care in Switzerland and across the globe.

Meet the researchers

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Professor Dr André Fringer completed his postdoctoral degree in 2011 in the Faculty of Health, Department of Nursing Science, at the Witten/Herdecke University in Witten, Germany. At the Institute of Nursing at the FHS St.Gallen, University of Applied Sciences in St.Gallen, Switzerland, he went on to serve as scientific head of degree for the Master of Advanced Studies in Palliative Care programme, as well as both project manager and deputy director in the Institute of Applied Nursing-Science. Today, he is a Family-Centred Care Professor, alongside co-heading his university's Nursing Science Research Unit, and heading the MSc in Nursing course at the Institute of Nursing at the Zurich University of Applied Sciences in Winterthur, Switzerland. Professor Fringer is now working with collaborators to investigate end of life nutrition and the onset of familial caregiving.

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A HOLISTIC APPROACH TO HEALTH NECESSITATES A DEEPER UNDERSTANDING OF HUMAN DEVELOPMENT

Connecting body and mind through the consideration of both the physical and psychological components of health helps determine our reactions and developmental behaviour. Furthermore, the ways in which we achieve our optimal developmental potential manage how well we can adapt and cope with changes in our environment, deal with stresses in life and maintain overall well-being. **Dr Natalia Sira** from East Carolina University is improving patient care by taking a holistic and individualised approach to health outcomes, treatment and rehabilitation, focusing on the role of family relationships, developmental needs and spirituality as important components of coping mechanisms.

Human Development and Health

Human development is a part of the larger, interdisciplinary field of developmental science in which the disciplines of biology, psychology and neuroscience have joined forces with education, medicine, and public health. To describe the vast interdisciplinary study of human constancy and change, development is divided into three broad domains: physical, cognitive and socioemotional in the description of developmental progression throughout the lifespan. This approach seeks to identify and explain the factors that influence the consistencies and changes in people over time, starting with infancy through to childhood, adolescence, emerging adulthood and finally, adulthood.

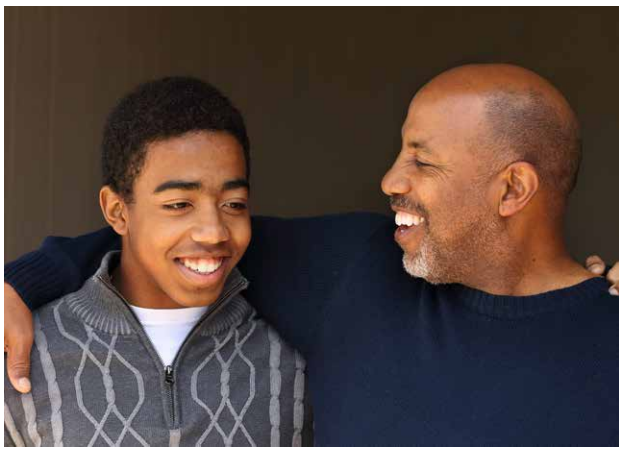
Physical development (growth and changes in the body and brain), cognitive development (learning, language and thinking) and psychosocial development (emotions and social relationships) are encompassed to provide an integrated, combined view. Each domain of development is influenced by other domains, creating a holistic and dynamic approach to understanding the living, growing and developing individual.

During the first five years of life, a child's brain grows in response to human interactions and emotions laying the foundation for health and future development. In response to the quality of caregiving, brain neurophysiology becomes wired and calibrated, providing the major structure for emotional control and self-regulation, responses to stress, bonding and separation, perceptions of self-worth and care. These are all connected with the survival centre that regulates autonomic functions (breathing, heart, and so on) that sustains life and other brain areas.

The neurophysiological responses to early experiences offer a blueprint for each individual, providing the internal

resources that underlie reactions to stress, quality of bonding, and perceptions of competency, hope and mastery over situations. These psychological resources also play a role in shaping behaviour, adaptation and coping when we face developmental transitions and stressors (such as body change and body weight, media/social pressure) and other stressors in life (including medical conditions and life-threatening illnesses).

To further understand and improve human health outcomes, it is vital to recognise developmental stages as well as age-appropriate challenges and behaviour. Viewing illness as a purely biomedical or physical problem is



insufficient, and without addressing developmental needs, in addition to socioemotional aspects of illness and its impact on the individual, effective patient care, support and prevention are impossible.

In other words, human functioning and health must be viewed holistically. While health triggers and stressors can be genetically or environmentally generated, our reactions to these triggers involve adjustments at both the body and mind (perceptions, emotions, reactions to stress) levels. This means that to be effective in medical methods, we must understand and reach for the internal psychological resources of individuals and include psychological support such as counselling, therapy or/and developmental experts, to help in recovery and to restore balance to our well-being.

Dr Natalia Sira at East Carolina University focuses on understanding human development, behaviour and functioning in a way that comprehensively considers influences on both the body and mind, and the implications of this on overall human health. Her specific areas of research include the role of family environment and early relationships on dieting practices, body satisfaction and self-esteem, coping and stress management. She conducts specific investigations into internal psychological resources, particularly for individuals in difficult circumstances such as those living with cancer or a chronic medical condition.

Body Satisfaction and Healthy Weight

Our body image or satisfaction with our physical appearance is influenced by biological, psychological and sociocultural components. Body satisfaction has been established as an important aspect of self-esteem and mental health, and is closely linked to an individual's perceptions and identity. Discrepancies often exist between an individual's true and ideal body image, which can lead to excessive dieting and other unhealthy behaviours.

Body dissatisfaction and disordered eating are currently viewed as a continuum ranging from eating disorders with related distorted body perception at one end to overeating and obesity at the other. Regardless of where one falls on this continuum, personal negative self-evaluation leads to body dissatisfaction and is linked to depression, anxiety, and overall, negatively influence the quality of life.

Since completing her doctoral dissertation on body satisfaction and weight, Dr Sira's research throughout the years has expanded upon this topic, with a critical focus on how parental attitudes, especially bonding (formed in early childhood as a blueprint for other reactions), are linked to body perception and satisfaction.

Dr Sira has identified that despite gender-specific associations, higher body satisfaction in males and females is found in those who report a higher degree of care (i.e., secure attachment/bonding) to their parents. Such an attachment is thought to provide a base for children to develop their sense of competency, including adaptation and coping, and contributes to self-esteem and resilience later in life. However, higher maternal control, which is thought to contribute to a child's inability to develop independence, can lead to dieting and is also associated with lower body satisfaction.

Dr Sira proposes that the quality of parent-child attachment relationships is an important factor in body satisfaction due to being linked to self-worth, competency and self-perception. A strong (secure) parent-child relationship is linked to the development of a healthy body perception, whilst insecure attachment and low self-esteem are common in those with eating disorders or body dysmorphia.

Dr Sira has shown that many risk factors for body concerns are linked to the family context, and they must be considered when promoting healthy body perceptions and eating habits. Triggers for eating disorders vary, which means that treatment for individuals must be tailored accordingly. This presents a significant challenge considering that many sufferers believe they do not need help, and their distorted perception of beauty is culturally orientated, deep-rooted and difficult to change.

Stress, Coping and the Role of Spirituality

Early emotional interactions with others and early childhood experiences influence brain development and shape the foundation for future health, reactions to stress and behaviour. Family relationships for each child early in life provide a sense of security that helps them to explore and relate to their environment. Without quality in care and familial structure, a child may not develop a sufficient sense of security or possess the internal psychological resources required to be equipped to feel competent and manage stresses later in life.

In research published in 2014, Dr Sira and her team investigated how the stress and anxiety induced in parents as a result of their child possessing a chronic heart defect can exacerbate negative health outcomes for the child. The entire family

dynamic is modified by how the parents cope with stress. More specifically, children whose parents cope effectively with their child's illness recover at a faster rate and have better health overall. Dr Sira concluded that supporting parents to cope by utilising psychological and social resources, encouraging behavioural strategies that are adapted to the differing needs of both mother and father, and most importantly, maintaining family integration and communication, is critical to aid the coping and recovery of the unwell child.

In more recent research, Dr Sira and her team investigated the coping mechanisms of emerging (aged 19–29) and young adult (aged 30–39) cancer survivors, with a focus on family bonding and spirituality. While coping is a complex skill depending on the psychological internal resources of an individual including self-regulation and competency, age also plays a key role in the ability to cope with multiple stressors. While (emerging) adults are often challenged in coping with developmental tasks in addition to cancer-related stressors, young adults have more life experience in dealing with more than one stressor at a time. Although overcoming the challenges associated with cancer at a young age has shown to contribute to quality of life and resiliency later in life, sadly, only 47% of cancer survivors cope well, accept or adapt to life with cancer. Instead, many fall into depression, self-blame or use dangerous distractions such as substance or alcohol abuse.

Dr Sira found that higher spirituality reliance, higher degrees of parental care or bonding and lower levels of parental control were associated with healthy coping in emerging and young adult cancer survivors. While spirituality is a part of life's philosophy, individuals rely on their spirituality to retrieve feelings of comfort, acceptance and hope, and in this case, reducing the likelihood of unhealthy coping mechanisms. Spirituality was found to represent the strongest contribution to healthy coping among young cancer survivors, yet spirituality as a coping tool remains somewhat unrecognised in the medical field.

Dr Sira argues that encouraging family support at the same time as encouraging independence, rebuilding supportive relationships, and recognising spirituality reliance as a coping tool should be incorporated into the recovery and treatment of young adults with cancer. Specifically, survivorship programmes and peer support groups to connect survivors and share experiences can help young individuals with their identity formation and self-discovery. Dr Sira emphasises that this approach will require a deeper and more sensitive understanding of the complex definition of spirituality, and what the term means to specific individuals. However, such an understanding would facilitate the recovery and rehabilitation of unwell individuals and their families in a way that considers both physical and psychological well-being.

In another study, Dr Sira investigated developmental outcomes of treatment-induced infertility among childhood cancer survivors. About 60% of children suffer from the effects of cancer treatment later in life, with more than 30% subsequently experiencing infertility, which adds further developmental challenges for young male and female cancer survivors. At a time in their lives in which they are forming personal identity through education, establishing intimate relationships, constructing a unique world view and considering parental roles, cancer survivors affected by infertility need to re-evaluate this traditional progression into parental roles and adulthood. Navigating an uncertain future, challenges to intimacy and restructuring identity can leave survivors with a sense of isolation. In this way, the developmental process of self-discovery, while building intimate relationships and family, becomes more complex through the added challenges stemming from infertility. This growing population of young survivors is deserving of care that extends beyond the elimination of disease.

Patient Care Quality

The quality of patient care relies on medical professionals such as nurses, doctors, child life specialists and occupational therapists. Developmental aspects of care should be present not only in paediatrics but also be extended to adolescence and emerging adulthood, for example, to encompass specific age-appropriate requirements incorporating the cognitive and socioemotional needs of the demographic. Dr Sira emphasises the importance of medical professionals considering educational, emotional and sociocultural tasks of the individual in the provision of tailored treatment, in addition to age and assumed maturity.

In research published in 2010 and 2016, Dr Sira found that supporting medical teams themselves will improve the care they give to patients. Secondary traumatisation, such as compassion fatigue, burnout and traumatic stress can affect health care providers, and this can influence their decision-making, empathy levels, behaviour and overall life satisfaction. This also can impact the patients they serve, which highlights why holistic care must be expanded to everyone involved.

Looking to the Future

Dr Sira now hopes to use her findings to support clinical services and advance the training of the young generation of professionals working in the medical field by advocating for developmentally oriented medicine encouraging the connection of body and mind in treatment and recovery/rehabilitation. This important work will pave the way for individually oriented and developmentally sensitive patient care.

Meet the researcher

Dr Natalia Sira
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Dr Natalia Sira received her medical degree from Uzhhorod State University in Ukraine and worked in oncology for a further 13 years. In 2003, she received her PhD in Human Development from Virginia Polytechnic Institute & State University, USA. She made the move to East Carolina University (also USA) in 2004, where she currently serves as an Associate Professor. Dr Sira's research is focused on understanding human health, and behaviour in a holistic and systemic way that considers the role of both mind and body, utilising both her medical and human development research background. She actively participates in professional societies such as the National Council on Family Relations and the Society for Studying of Emerging Adulthood. Among a multitude of other awards, Dr Sira received an Outstanding Professional Paper Award from the National Council on Family Relations in both 2013 and 2018.

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DRIVING FORWARD A PARADIGM SHIFT IN HEALTHCARE: THE MEIKIRCH MODEL

Healthcare systems around the world are under increasing pressure as a result of depleting resources accompanied by rising demands. **Dr Johannes Bircher's** reflections on the inherent failings of modern healthcare systems have coalesced into an important proposal for a new concept of health, known as the Meikirch model. Here, we look at the potential of the Meikirch model to drive forward a critical paradigm shift in healthcare delivery.

An Unsustainable Healthcare System

Dr Johannes Bircher has had a distinguished and notable career in medicine. Reflecting on his impressive more than 50 years of experience, Dr Bircher has concluded that healthcare systems are unfit for purpose, unable to respond adequately to the rapidly changing expectations of the public and government and the financial pressures being faced at every level of administration. He notes that healthcare costs are spiralling, but conversely, financial constraints are requiring healthcare managers to make significant cuts in services and staffing, a scenario he describes as 'unsustainable'.

The Meikirch Model

In his analysis of the current state of healthcare, Dr Bircher has identified the core problem as being focussed on the concepts of health versus a medical system focussed largely on disease and illness.

To articulate and propagate his ideas, Dr Bircher has collaboratively developed the Meikirch model, with co-authors Dr Shyama Kuruvilla and Dr Eckhart Hahn. The model presents a new conceptual framework for our understanding of health and disease, and a resulting paradigm shift for healthcare systems, that will be required if our healthcare systems are to become sustainable and more effective. Dr Bircher describes the Meikirch model as, 'not only a unifying theoretical framework for health and disease but also a scaffold for the practice of medicine and public health'.

A New Framework for Understanding Health

At its core, the Meikirch model proposes 'health' to be a person's capacity to fulfil the demands of life within the context of their social and environmental circumstances.

Each individual receives the 'gift' of a 'biologically given potential' at birth, although, to a large degree, this is a lottery of fate. Our genetic make-up and time in the womb may impact positively or negatively on this potential. Once born, our biologically given potential is finite and diminishes over time until our death.

The demands of life change from birth to old age. As new-born babies, we are completely dependent on others for care, but as we grow, our resources to cope with the demands of life grow with us. We acquire skills, knowledge and attitudes to (generally) cope with the situations we face, although disease and external factors that are outside of our control may mean that we require additional support. As we near old age, we again become more dependent on external care, as the demands of ageing and illness overwhelm our coping capacity.

In balance with our biologically given potential, the Meikirch model proposes that we also develop a 'personally acquired potential' as we mature. This may be conceived as the life-skills, personality development, behaviours and attitudes we learn, adapt and utilise to maintain a 'healthy' life and to cope with the demands that life throws at us. The model envisages that 'personally

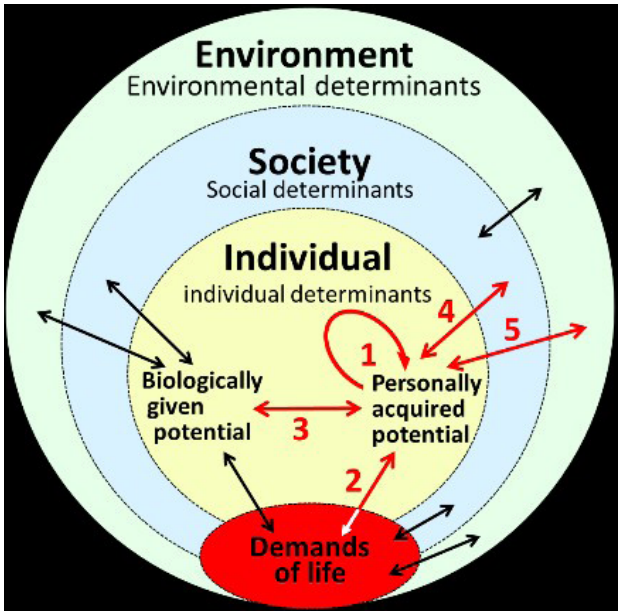

Figure 1. Schematic representation of the Meikirch-model (taken from the first reference on the meet the researcher page) The red numbers in the figure represent the five spheres of personal responsibility for health as explained below.

1. Responsibility for inner development or maturation: Meditation, group therapy, retreats, psychotherapy, and so on.
2. Responsibility to make sure that the demands of life are appropriate, not excessive but also not too little. They should be challenging in a measure that is right for the person.
3. Responsibility for the biologically given potential, i.e., for the physical body. Too many people are overweight, drink too much alcohol or are still smoking. A balanced diet and physical exercise are important.
4. The society is the sum of humans who deal with a person, starting with the mother, then the family and then the society. Each person should participate and live in a culture of love and forgiveness and support for other persons. This has to be cultured during the whole life. Children should be allowed to grow up free from overpowering but with support to develop their personal skills and capacities.
5. The responsibility for the environment is much in the news but is not realised by all (people with large cars, for example).

acquired potential' generally grows as biological potential recedes.

The Meikirch model describes our potential for health as the sum of our biologically given and personally acquired potentials. The contribution of each potential alters throughout the life-course for every individual, and any response to an adverse health event will involve both potentials. However, where an individual adopts a self-damaging behaviour, such as through alcohol or drugs, then their personally acquired potential that may have already been reduced will be diminished further by the inappropriate behaviour, finally reducing their ability to cope with the life demands that subsequently occur.

The Implications for Healthcare

When developing the Meikirch model, Dr Bircher sought to carefully consider the implications of the model on healthcare systems and practice, based on the fundamental ideas that underpin it.

Primarily, the model questions the dominant role of traditional, disease-focused medicine as the most sustainable approach to improving an individual's health. Public health and health promotion practitioners see health within a holistic context, where the addressing of environmental and social determinants of health are perceived as essential elements in improving an individual's health. The Meikirch model acknowledges these contextual factors as important influences in one's health potential and describes the model as a 'complex adaptive system', a matrix of interconnected influences (demonstrated by the arrows in the model's graphic representation – see Figure 1).

These contextual factors, however, are often neglected in traditional illness-focussed medicine, where the individual's disease is treated in isolation from their personally acquired potentials, and how these might resist or assist their recovery to health. Remembering that 'health' here is defined as the ability to cope with life's demands, that may mean that the person may be able to live adequately with a disease or long-term condition (e.g., diabetes mellitus) and be considered 'healthy', if supported to increase their necessary personally acquired potential to do so. In practice, this may mean additional psychological or emotional support, assisted living adaptions, or improved financial support to cope with the loss of income, and so on.

Dr Bircher believes that using the Meikirch model can be an effective way to reframe the common goals of the various healthcare disciplines and professions. He notes that healthcare practitioners often have differing views of what constitutes a 'health' outcome, which can result in a conflict of interest, a fight for resources and ineffective coordination. He further states, 'It is our hypothesis that the Meikirch model offers much more precise objectives for the division of labour in the interest of a joint purpose…The Meikirch model will thus allow a more rational exchange of opinions about every problem, possibly leading to better solutions and resulting in significantly improved cooperation.'

Dr Bircher emphasises the need for a shift to a person-centred healthcare system and away from the value-based payment-driven healthcare systems that dominate most of the Western world, with purely materialistic business-driven values at their heart. He argues that this approach undervalues and neglects integrative medicine and lifestyle improvement approaches and is inadequate to address the patient's personally acquired potentials.

The Consequences for Health Promotion

Another fundamental aspect of the Meikirch model is the recognition that, through the concept of the personally acquired potential, the individual must take greater responsibility for their own health and maximise this wherever possible.

Dr Bircher uses the example of self-care in type 1 diabetes, where a person can be trained and motivated to monitor their own blood glucose, inject insulin, maintain an appropriate diet and take adequate physical activity to improve and maintain their future health.

Responsibility for one's own health also extends to more holistic approaches. For example, the personally acquired potential can be enhanced through mind-body approaches such as meditation and mindfulness, which have been shown to be effective preventative treatments for many chronic conditions such as migraine and different pains, alongside medical or pharmacological treatments, but also increase general well-being and longevity.

By expanding social and environmental support to utilise self-treatment options, there is great potential not only to improve people's health but also reduce healthcare costs considerably, by reducing reliance on expensive medical treatment that often may be no more effective than non-medical therapies.

The Model in Practice

The Meikirch model has been put into practice in a small project led by Sarangadhar Samal of the National Youth Service Action and Social Development Research Institut Nysasdri in Bhubaneswar, India.

In 2013, Sarangadhar was involved in social work with tribal villagers to improve their lives and health. Dr Bircher developed a manual for the Meikirch model which was translated into Orio, the local language, for use in 40 'test' villages. The villagers learned about the new definition of health with the focus on self-responsibility (a new approach for the region) and identified the demands on their lives and the resources available locally to deal with these in the present and future. The project assessed whether the model would influence the villager's health behaviour in comparison to neighbouring control villages.

Issues identified as 'demands on life' by the villagers included eating a balanced diet, adequate housing to protect from the rainy season and wild animals, good hygiene, child vaccination, sexual disease prevention, maintaining a happy family life, avoiding superstition and irrational beliefs, and good communication within and among other villages.

After 30 months, the project showed significant improvements in health-related behaviour, although it was acknowledged that it could still be improved further. The practical effects of teaching the Meikirch model included improved outcomes on household nutrition, the wearing of slippers in latrines, washing hands before meals, childhood vaccination, use of mosquito nets, good attention to childcare and the number of household latrines.

Future Implications

For a paradigm shift in practice and beliefs to occur, it can take many years before it becomes an established norm. For the Meikirch model, Dr Bircher notes that it has taken 20 years for it to become established and discussed as a viable way forward.

The model fits well with new 'omics' technologies (e.g., genomics, epigenomics, proteomics) which, in the future, will enable highly personalised analysis of an individual's genetics, cause of disease or illness and their best, specific treatment options. In this way, an individual's biologically given potential can be assessed and boosted as part of an integrated package of medical and public health measures.

The model also has the potential to dramatically change the patient-doctor relationship. Greater self-responsibility for one's own health removes the 'benevolent paternalism' that defines the present relationship, and doctors will need to become empathetic partners to support and broker therapy for the patient to improve their personally acquired potential. In doing so, Dr Bircher believes this will break down some of the more irrational therapies of alternative and complementary medicine. In contrast, it will strengthen personal and emotional development.

The Meikirch model, as a new conceptual framework of health, impacts upon many aspects of medicine and public health. Its ramifications for change are enormous and while far from easy to implement, could address many failings of current healthcare systems and let them into a better future. The focus on a patient-centred and holistic approach embedded into their specific social and environmental circumstances results in a highly appealing model that now should be urgently tested.

Meet the researcher

Dr Johannes Bircher

Born in 1933, Dr Johannes Bircher studied medicine at the Universities of Lausanne, Switzerland, Munich, Germany and Zürich, Switzerland. His professional career in internal medicine continued at the Mayo Clinic in Rochester, USA, and the University Hospital in Zürich. From 1975 to 1989 he was Associate Professor at the University of Bern, Switzerland and Full Professor at the University of Göttingen, Germany, both in the Department of Clinical Pharmacology. He concluded his formal career as the Dean of the Medical School of the University of Witten/Herdecke, Germany and as the Director of Medical Services at the University Hospital in Bern, Switzerland. Dr Bircher is known as an author and editor in scientific medical literature and is an Honorary Member of the Swiss Academy of Medical Sciences.

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INVESTIGATING HEALTH PROFESSIONALS' RETIREMENT DECISION-MAKING

The availability of skilled health practitioners is fundamental to the health of a nation. Unfortunately, many countries experience shortages of healthcare professionals. Shortages typically result when too few choose to enter the health professions and when too many exit the health professions, whether to pursue an alternate career or to retire. **Dr Sarah Hewko**, based at the University of Prince Edward Island, is conducting valuable research into the reasons why health professionals retire earlier than planned.

Ageing Populations and Healthcare

Even before the COVID-19 pandemic, health systems around the world were struggling to cope with increasing demands for medical care. In keeping with a trend that first presented in developed countries, nearly all countries are now having to meet the needs of an increasingly ageing population. This has significant implications for healthcare, as older adults tend to access health services more frequently. Furthermore, declining birth rates have led to there being fewer new entrants into the labour market. In a nutshell, more people are needing healthcare but there are fewer skilled professionals available to provide it.

The availability of skilled health practitioners is affected both by the number of individuals who enter the health professions (which depends, at least in part, on the availability of places in profession-specific training programs) and by the number of individuals who leave those professions, whether to retire or to pursue alternate employment.

Healthcare Professional Shortages Across the World

The Global Health Workforce Alliance and the World Health Organization recently reported a global deficit of skilled healthcare professionals of around 7.2 million – and this number is increasing. The consequences of such shortages vary but include increased costs of healthcare, increased wait times for health services, and longer working hours for existing staff.

Shortages in healthcare are present across professions, including in medicine, nursing, and the allied health professions. Allied health professionals (AHPs) include, among others, dietitians, pharmacists, physiotherapists and speech and language therapists. Unfortunately, the causes of workforce shortages within the allied health professions are under-researched. In particular, little is known about the factors influencing retirement in these professional groups.

Researching Retirement

Dr Sarah Hewko, Assistant Professor at the University of Prince Edward Island, has been researching how healthcare professionals approach retirement decision-making retirement for several years. In a key study, Dr Hewko utilised baseline data from the Canadian Longitudinal Study on Aging (CLSA). The CLSA is a national study which will follow approximately 50,000 Canadians, aged between 45 and 85 at the time of recruitment for 20 years. The CLSA is one of many databases across the globe tracking population-level outcomes of ageing.

Although the average age of retirement in Canada is 64, Dr Hewko found that AHPs were retiring, on average, between 56 and 60 years old (depending on

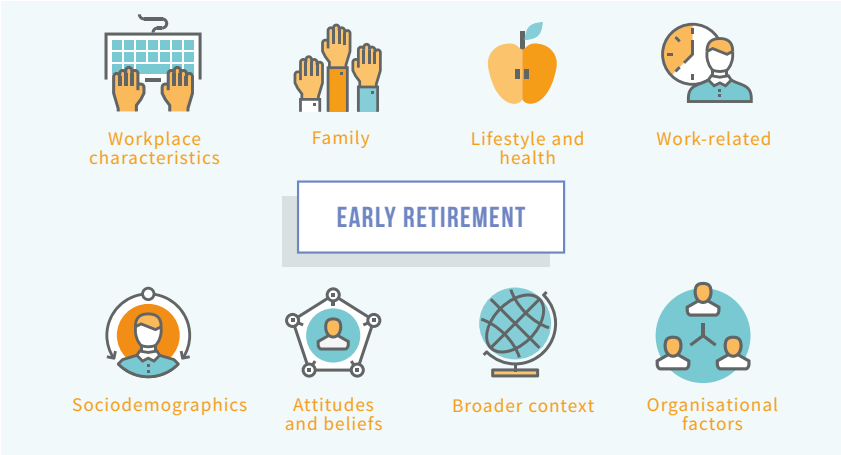

Figure 1: Conceptual model of early retirement among registered nurses and allied health professionals.

profession) with RNs retiring at 58 years old. With early retirement defined as retirement below 65 years of age, Dr Hewko found that not only were many AHPs and RNs retiring 'early' but, in some cases, they were retiring earlier than they had planned. Her next step was to identify the reason(s) why.

Dr Hewko and her colleagues began by reviewing the available published research. From this, they developed a conceptual model of the factors most often reported to affect early retirement decision making in RNs and AHPs. A conceptual model is essentially a visual representation of key factors (or variables) proposed to influence an outcome – in this case, early retirement.

Conceptualising Early Retirement

In Dr Hewko and her colleagues' conceptual model, factors proposed to affect early retirement were identified based on previously published findings. These fell into eight broad categories (as shown in Figure 1).

Workplace characteristics included whether older workers were recognised and valued in the workplace, the frequency of structural and/or technological change, and the flexibility of working hours. Age-related stigma and discrimination can be characteristic of a workplace and may cause health professionals to retire early if, as they age, they come to view the workplace as an increasingly unwelcome space. Other work-related factors included work schedule (e.g., days/nights, rotating shifts), how long the individual had been working over their entire career, and how long they had been working for the current organisation.

Sociodemographic factors, such as educational level, financial security and childcare responsibilities also featured in the model. For example, healthcare professionals who perceive themselves as financially secure may decide to retire early as they can afford to support themselves without working. The related category of family incorporated factors such as whether one's partner was retired, the partner's health status, and caregiving responsibilities, such as caring for elderly parents.

Lifestyle and health-related factors are also critical to decisions surrounding retirement in later life. Through the process of ageing, our health may begin to deteriorate to the point where working is no longer practical. As such, physical and psychological health status, level of physical activity and the presence of any disability will affect an individual's ability to work and be a significant contributing factor to the decision to retire. Societal expectations may also lead individuals to retire early (or earlier than planned) as others begin to perceive them as 'infirm' or less physically capable (even if their performance remains unchanged).

A healthcare professional's attitudes and beliefs around work and retirement also influence retirement decisions. Job satisfaction is a key element of this, as is the individual's level of commitment to the organisation and/or their occupation. Personal views of retirement are also important, alongside an individual's desire to have more time for leisure activities.

Across all occupations, the broader context of age, within the context of 'generations', is a much-discussed factor that may influence the timing of retirement. The generation known as 'baby boomers' (born between 1946 and 1964) reached 65 years beginning in 2011 and the subsequent 'generation x' is the next to face retirement.

Another factor was the 'location of residence', which is associated with differences in legislation, taxation and labour laws across municipalities, provinces, states and districts. Characteristics and policies of the employing organisation also affect the decision to retire. Incentives to leave for early retirement may be featured during planned organisational restructuring, increasing the appeal of early retirement for the individual.

We can see that many factors proposed in the model interact with others, both within each category and across categories. For instance, the flexibility of working hours (which falls under 'workplace characteristics') may affect whether or not a healthcare professional decides to continue working alongside caring for older family members (a factor considered under the category of 'family').

The conceptual model of early retirement was validated for clarity, logic and comprehensiveness by Dr Hewko through a series of interviews with Canadian RNs and AHPs. Interviewees confirmed that the model was clear, logical and included all the factors relevant to early retirement among health professionals.

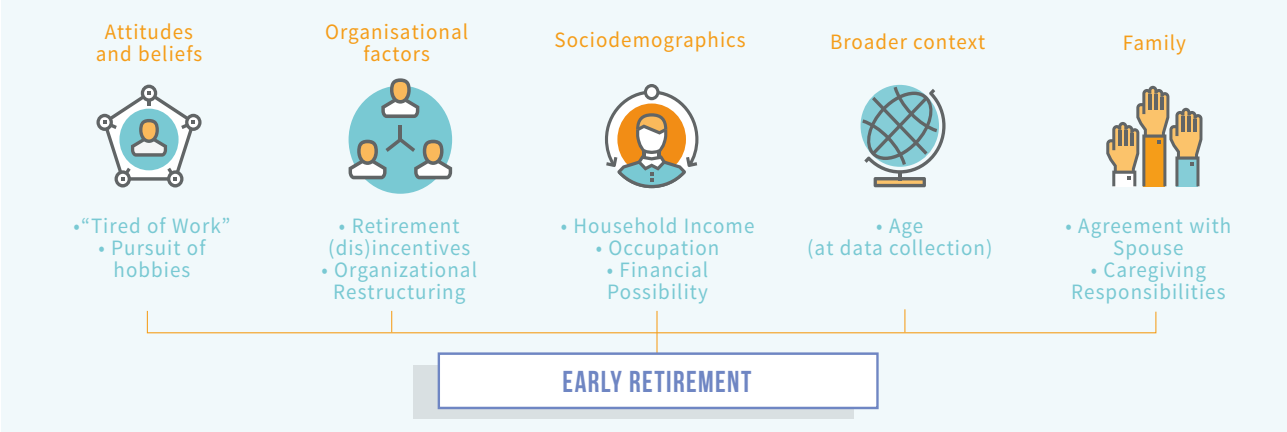

Figure 2: Analytic model of early retirement among registered nurses and allied health professionals. Adapted from S Hewko, et al., The early retiree divests the health workforce: a quantitative analysis of early retirement among Canadian Registered Nurses and allied health professionals, Human Resources for Health, 2019, 17, 49, under the Creative Commons Attribution 4.0 International License.

Application of the Conceptual Model to Data

Following the validation of the model, Dr Hewko tested its ability to account for early retirement in RNs and AHPs, drawing again on data from the CLSA. Using a pared down version of the model (see Figure 2), the categories of 'attitudes and beliefs', 'organisational factors', 'sociodemographics', 'age' (broader context) and 'family' were able, to some extent, to (statistically) explain the real-world data for both RNs and AHPs. This demonstrated the utility of the model.

Of the specific factors of interest, Dr Hewko identified that both RNs and AHPs reported organisational restructuring as influencing their decision to retire early. For RNs, further significant factors including early retirement being financially viable, profession of a desire to stop working, and caregiving responsibilities.

Proposed Avenues of Change

Given the urgent shortage of healthcare workers and the healthcare requirements of the increasingly ageing population, there is a clear need to retain healthcare workers in the workforce for as long as possible. Extending the work-lives of RNs and AHPs by an average of even 6 months each could have a significant impact on national workforce shortages and the sustainability of health systems.

Organisational restructuring often occurs in response to budget restrictions and can involve the elimination of certain roles, as well as changes to reporting structures. Older workers may be more affected by restructuring as they tend to be on higher salary bands and therefore cost organisations more. Older workers, particularly those who have been with the employer for a long time, are significant sources of organisation-specific historical knowledge and are of significant value in the efficient and effective training of newly hired professional staff and student health professionals. As one of the few work-specific factors identified as influential to retirement decision-making by both RNs and AHPs, it is, fortunately, one of the easiest to address.

Dr Hewko recommends effective and frequent communication throughout organisational restructuring as a method to lessen uncertainty that might contribute to healthcare professionals opting for early retirement. She also recommends stronger collaborations between trade unions and hospital management to avoid adverse impacts resulting from restructuring.

Critically, for RNs, caregiving responsibilities were associated with 7.2 times greater likelihood of early retirement. This is perhaps unsurprising given that women are, in addition to their vocation, likely to also fill the stereotypically nurturing roles of mother, wife and daughter. Noting this, Dr Hewko argues for greater implementation of caregiver-friendly policies to encourage healthcare professionals to remain in the workforce while caregiving and to facilitate penalty-free short-term exits from employment to fulfil time-limited needs for full-time caregiving, such as during cancer treatment regimes, post-surgery or at the end-of-life.

Dr Hewko's work has highlighted the interrelated and often complex factors that contribute to a healthcare professional's decision to retire at a certain point in their lives. With support from the Ireland Canada University Foundation, she will be travelling to Ireland in the coming year to conduct analyses looking at similarities and differences in retirement decision making among registered nurses (RNs) and AHPs in Ireland and Canada.

As such, Dr Hewko's further research will continue to provide knowledge that will be key to building and maintaining sustainable healthcare systems. Through understanding the reasons why people retire early, efforts can more effectively be focused on retaining more healthcare professionals for a longer period of time and addressing health workforce shortages in Canada and beyond.

Meet the researcher

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Dr Sarah Hewko obtained her Master of Health Administration from the University of British Columbia before going on to the University of Alberta to complete a PhD in Nursing in 2018. Trained as a dietitian, she has worked as a manager of clinical dietitians and maintains a small, specialised practice where she treats clients struggling with disordered eating. Dr Hewko currently holds the position of Assistant Professor in the Department of Applied Human Sciences at the University of Prince Edward Island. In her research, she focuses on dietetics, allied health workforce planning, and healthcare administration. Dr Hewko's work has been published in *Human Resources for Health, Healthcare Policy, Leadership in Health Services and Health Policy* (among other peer-reviewed journals). In addition, she has presented her research at conferences across Europe and North America.

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UNDERSTANDING AND ENSURING THE PROVISION OF SAFE DRINKING WATER

Water is vital to sustaining human life and the contamination of drinking water can lead to disease and death. **Dr Steve E. Hrudey** from the University of Alberta's Division of Analytical & Environmental Toxicology has identified the challenges of providing safe drinking water and clarified misconceptions regarding threats to drinking water safety. Based on his research findings, he has provided critical recommendations for the provision of safe drinking water to protect public health.

The Importance of Safe Drinking Water

In 2010, the United Nations General Assembly recognised the human right to sufficient and safe water because it is essential to life. However, water is not naturally safe to drink. Over the past two centuries, the role of fully natural microbes (microscopic organisms such as bacteria, protozoa and viruses) in causing disease has been documented. Drinking water contaminated with microbial pathogens has been conclusively established to be the major cause of disease outbreaks arising from drinking water.

To be made safe, drinking water must be treated, managed and delivered to the point of use to reduce the risk of contamination to that of negligible likelihood. In some less developed countries, there is a lack of adequate drinking water services, resulting in contaminated drinking water which increases the risk of enteric diseases such as cholera, dysentery and polio. According to the World Health Organization, more than 800,000 people are estimated to die each year from diarrhoea caused by unsafe drinking water.

Affluent nations are fortunate to have the resources to provide safe drinking water and, therefore, experience fewer deaths associated with drinking water-borne diseases. However, despite having mostly advanced water management systems, drinking water disease outbreaks continue to occur in rich countries. Such outbreaks are clearly a preventable threat to public health.

Dr Steve E. Hrudey from the University of Alberta has examined the evidence surrounding drinking water disease outbreaks in affluent countries. In doing so, he has identified common underlying causes of such outbreaks, clarified common misconceptions about what safe drinking water means, and provided recommendations for ensuring the provision of safe drinking water.

Challenges to Providing Safe Drinking Water

In a report prepared for the Canadian Water Network, Dr Hrudey outlined the challenges that drinking water providers face in the effort to supply safe drinking water. Understanding what constitutes 'safe' drinking water is necessary to

minimise human health risk. However, what is deemed safe by health experts may not satisfy consumers' concept of safe drinking water. Drinking water providers must understand public expectations of safety to maintain consumer trust.

While the concepts of safety and risk can vary according to context and individual perception, Dr Hrudey adopts a pragmatic approach, defining safety as 'a level of risk that is so low that an accurately informed person need not worry about it.' Everyone who is born is certain to eventually die. What is not certain is when and from what causes. There is an enormous range of health risks that may shorten anyone's life, far too many to all be treated as equally important for any one person. 'Safe' drinking water poses a non-zero, but truly negligible risk to human health.

‘Documenting case studies of drinking water outbreaks in affluent nations reveals that they are fundamentally a result of complacency and a failure to do what we know how to do.’

E coli *bacteria*

Conventionally, safe drinking water has been pursued by setting guidelines for individual parameters, such as maximum levels of specific contaminants, on a precautionary basis to seek negligible heath risk over a lifetime of human consumption. While the simplification of the problem in this manner makes it seem more manageable, there are several challenges to this approach.

For example, a challenge for drinking water regulators arises when specific water quality monitoring yields detections approaching or exceeding these precautionary guideline values or where guideline values do not yet exist for particular contaminants. Additionally, some lower limits for contaminants have been based on an arbitrary detection limit rather than a well-informed health risk assessment. These may create public concern that is not based on credible evidence of health risk. Because many concerned individuals do not appreciate that detection of contaminants in water can and frequently does occur at levels far below what is necessary to cause adverse health effects, such trace detections can create unwarranted worry. Furthermore, monitoring water for a long list of contaminants, most of which are unlikely to pose a credible risk, can drain resources from operational issues of greater value to ensuring safe drinking water.

A key challenge to the provision of safe drinking water is a misunderstanding surrounding what threats are most important and in need of action. As Dr Hrudey explains, ‘the greatest risks to human health from public drinking water supplies in developed nations arise from microbial pathogens in faecal wastes from humans, livestock or wildfowl/wildlife.’ However, there is often higher public concern regarding the detection of chemical contaminants in drinking water at negligible concentrations and showing uncertain epidemiological evidence and inadequate relevant toxicological evidence. Though disinfection is a necessary process for removing pathogenic microbes from drinking water supplies, there is often opposition to disinfection driven by concerns about the resulting chemicals known as disinfection by-products.

In reality, as Dr Hrudey notes, recent fatal outbreaks caused by contamination of drinking water in affluent nations have been caused by microbial pathogens rather than chemicals. During and since his participation in the public Walkerton Inquiry (2000–2002), Dr Hrudey has focussed on studying the causes of drinking water outbreaks in developed countries to understand the common failures that lead to such outbreaks.

Past Mistakes and What They Teach Us

In May of 2000, heavy rainfall washed livestock manure from a nearby farm into a town well, contaminating the water supply to Walkerton, Ontario, Canada. Inadequate disinfection of the water resulted in an outbreak caused by the pathogenic *E. coli* O157:H7 bacterial strain and *Campylobacter* spp. that sickened over 2,000 individuals and led to seven deaths. The public Inquiry, led by Justice Dennis R. O’Connor, sought to establish the cause of the outbreak.

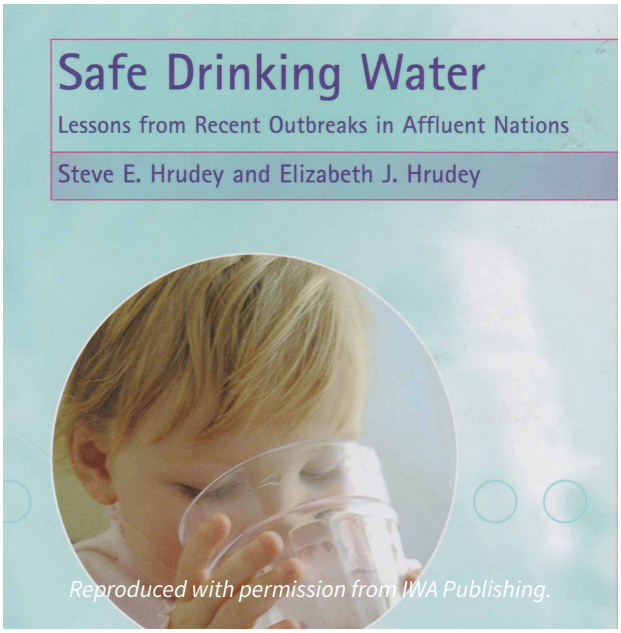
Since the Walkerton public health disaster, Dr Hrudey and his wife Elizabeth have published two international books documenting case studies of drinking water contamination failures. More than 30 major drinking-waterborne disease outbreaks in affluent countries have been reported in the scientific literature since Walkerton. The Hrudeys examined reports of these outbreaks and described the major recurring themes that caused such failures in a refereed journal article published in 2019.

First among the main themes identified were complacency, naivete and ignorance. Those responsible for delivering safe drinking water were found to have overlooked or misunderstood how prevalent faecal contamination of water sources is, as well as failing to recognise that livestock and wildlife host pathogens that can infect humans and that these hosts excrete these pathogens in their faeces.

Some of the cases that were reviewed involved simple failures such as not preventing human sewage discharges or livestock access from contaminating source waters. Therefore, despite knowledge afforded by decades of research into pathogens and their origins, well-characterised threats to drinking water safety had been overlooked in these outbreaks in affluent countries. As Dr Hrudey stated, ‘documenting case studies of drinking water outbreaks reveals that they are fundamentally a result of complacency and a failure to do what we know how to do.’

Another common issue identified by the Hrudeys was a failure to learn from experience. In these instances, individuals who were responsible for providing safe drinking water had not been informed about basic lessons from previous failures,

Credit. Steve Hrudey



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nor had they been adequately trained to recognise and avoid conditions that would allow preventable outbreaks to occur. This highlights a responsibility to communicate public health threats widely and effectively to all of those who are involved in providing safe drinking water.

Dr Hrudey has also found that fear of chlorination disinfection by-products has resulted in inadequate disinfection that contributed to waterborne disease outbreaks in many of the reported case studies. Although the human health risk of pathogenic microbes in drinking water has been well established in contrast to the uncertain risk from disinfection by-products that is generally negligible, there is a prevalent public belief that the chemicals pose a greater threat to public health than pathogens.

Recommendations for the Prevention of Drinking Water Disease Outbreaks

As a result of investigating recent drinking water outbreaks in affluent countries, Dr Hrudey concluded that such incidents were eminently preventable. This research identified the need for improved communication and education regarding previous outbreaks among all of those involved in providing drinking water. Dr Hrudey suggests that rather than relying primarily on compliance monitoring of contaminants, maintaining effective operations and implementing effective drinking water safety plans will enhance the capability of operational personnel to achieve better prevention of waterborne disease outbreaks. Drinking water safety plans must document a full understanding of one's water management system, the contaminant challenges it faces and the capabilities and limitations of the water safety barriers that are in place and are shown to be functional.

Drinking water safety plans must be living documents, fully understood by operators, used regularly and updated often. In accordance with this preventive risk management approach, Dr Hrudey suggests recognising a hierarchy of drinking water risks that can be adapted to local needs. Using this approach will ensure that the most critical risks receive the highest priority while less critical risks are given the less urgent level of attention they warrant. The first, most urgent category of risk denotes common, pervasive risks, highly certain to cause human disease, such as well-characterised microbial pathogens. Contaminants in this category require prompt and continuous effective action for any drinking water system.

The second, lesser priority risk category features reasonably certain but substantially less pervasive risks, such as lead, natural arsenic and excess fluoride, which should be identified where they occur and addressed as necessary. Common but comparatively uncertain and otherwise lesser risks in the third level of the hierarchy require a rational precautionary response. This third level includes disinfection by-products.

Finally, in the lowest risk category, site-specific contaminants with noteworthy toxic potential, such as pesticides, require localised plans appropriate to their risk. Such risk is typically low because this type of contaminant exposure through drinking water consumption is usually far too low to cause adverse health effects. This lowest risk category is also reserved for emerging contaminants that require research to characterise the nature of their risk.

Ultimately, Dr Hrudey advocates that the task of providing safe drinking water to the public requires collaboration and communication among all who are involved, utilising current knowledge regarding risks and employing effective preventive strategies. In doing so, the provision of safe drinking water can be ensured and risk to public health will be reduced.

Meet the researcher

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Dr Steve E. Hrudey obtained his PhD in Public Health Engineering in 1979 and later received his Higher Doctorate (DSc) in Environmental Health Sciences and Technology in 2002, both from the University of London. Currently, he is Professor Emeritus at the University of Alberta's Division of Analytical & Environmental Toxicology. Dr Hrudey's career in environmental and public health risk spans five decades, during which he has served on several expert panels dealing with high profile environmental health issues, published more than 190 peer-reviewed journal articles, and authored numerous books. Among other awards, Dr Hrudey was appointed a Member of the Order of Canada in 2019, received the Alberta Order of Excellence in 2017, and was recognised with a Queen Elizabeth II Diamond Jubilee Medal from the Royal Society of Canada in 2013. Dr Hrudey's research focusses on addressing misconceptions concerning public health and environmental risks with a major emphasis on safe drinking water.

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LINKS BETWEEN FRACKING AND A RARE BIRTH DEFECT IN RACEHORSES

Fracking has long been controversial for its potential to contaminate local water sources and cause damage to the environment. **Dr Kathleen Mullen** at Littleton Equine Medical Center in Colorado and her team have carried out novel research into how fracking in close proximity to a horse-breeding farm may be the cause of a specific birth defect in the foals born there. Her findings may be relevant to human babies and considerations of the implications of fracking on long-term health.

Fracking in the USA

Between the layers of the sedimentary rock, shale, natural shale gas can be found in areas called shale plays. Over millions of years, the shale rock is formed by layers of mud build-up compressing the levels below. If this rock is heated to 100˚C, which happens at least 2 km below the surface of the earth, shale gas is produced from the organic material. The layers are non-permeable, which means that gas cannot pass through, trapping it deep below the ground.

Shale gas is extracted using unconventional natural gas development (UNGD) methods, including horizontal drilling and hydraulic fracturing, commonly known as fracking. Horizontal drilling involves drilling a deep hole vertically into the shale formation, then horizontally to allow access to more gas reserves. This well is then filled at high pressure with fracking fluid made up of mostly sand and water, but also chemicals that prevent well corrosion and microorganisms from growing. The newly formed fractures in the rock allow the shale gas to escape upwards and it is captured in collection wells. The gas can then be processed and piped away, and the fracking fluid is collected and treated to remove contaminants.

The hydrocarbon methane makes up a majority of this natural gas. It has domestic uses, such as fuel for vehicles, heating, cooking and electricity, as well as being a component of plastic manufacturing. First extracted in Fredonia, New York in 1825, shale gas went on to make up a majority of natural gas produced in the USA after large-scale operations started in Texas in the 1980s. Its varied benefits made it appealing for investment in the USA.

Combatting climate change is becoming increasingly important. Supporters of UNGD argue that burning shale gas produces significantly fewer greenhouse gases than other non-renewable sources of energy. Some studies have found that, per unit of energy released, combustion of natural gas releases half as much carbon dioxide than coal combustion. This means that it burns 'cleaner' than coal or oil because less carbon dioxide, nitrogen oxide, sulphur

dioxide is emitted into the atmosphere. Local economies can also benefit from UNGD as employment opportunities are produced during a time when other non-renewable energy jobs are reducing.

The Downside of Fracking

Although fracking has positives, it is also highly controversial due to its potential to cause environmental damage and disruption. Areas where UNGD takes place may experience higher levels of noise pollution and road traffic and a significant amount of space is needed for the operation. The risk of earthquakes slightly increases however, mild tremors are more common. For these reasons, house prices have been known to fall surrounding new fracking sites.

One of the biggest risks of fracking is air and water contamination. Burning shale gas gives off pollutants such as polycyclic aromatic hydrocarbons (PAHs). PAHs are carcinogens, which means they can cause cancer, but they may also result in other health issues. If cracks form within the borewell or a spill occurs, wastewater could leak into the local water supply, creating huge health implications.

Around 350 of the 630 chemicals used in fracking fluid are known to be bad for your health. Exposure to these contaminants in the soil, water supply and air has adverse effects on asthma sufferers, people with cardiovascular issues and pregnant women. Particularly concerning is the link between proximity to a fracking site and new-born complications. Lighter babies, developmental issues, congenital defects and premature births are all associated with prenatal PAH exposure.

Exposure to Polycyclic Aromatic Hydrocarbons in Pregnant Horses

In 2014, while working at Cornell University Hospital for Animals as a veterinarian, Dr Kathleen Mullen and her colleagues diagnosed five foals with a birth defect called dysphagia from the same racehorse breeding farm in Pennsylvania, USA. What made this particularly interesting is that only ten foals were born that year and dysphagia is so rare, normally less than 1% of foals are born with it. Between 2012 and 2014, nine out of twenty-four foals born there had the defect.

Dysphagia is the inability or difficulty to swallow in horses. It can be acquired during adulthood, but in these foals, it was due to a congenital birth defect, meaning it had been present since birth. There are various reasons why foals may struggle to swallow its mother's milk including structural abnormalities such as cleft palate and functional abnormalities such as neuromuscular dysfunction. In this case, they found that the foals had no structural abnormalities present and they appeared more subdued than normal foals - lower mentation (mental activity). For these reasons, they suspected a congenital neurological disorder. Instead of ingesting the milk properly, the foals inhaled it, which is known as tracheal milk aspiration. As well as preventing the foals from getting much-needed nutrients, inhaling milk can result in bacteria growing in their lungs, leading to pneumonia.

The owners of the farm in Pennsylvania also own a horse farm in New York, USA. The NY farm had not experienced a single case of dysphagia in ten years. Both farms sourced their hay and grain from the same place, but crucially, their water and pasture differed according to their location.

Dr Mullen and her team noted that there were 28 fracking wells within 10 km from the Pennsylvania farm and two of them were less than 500 m from the water wells that supply water to the horses. However, in New York, the process of fracking is banned and so there was no fracking activity surrounding the second farm.

As a result, the team hypothesised that the UNGD operations and consequent chemical exposure around the Pennsylvania farm were linked to the foals being born with birth defects. They believed that this may be an important

example of how fracking may result in adverse health effects, perhaps even in human babies. The study the team conducted was the first to measure PAH levels in the air and water throughout a mare's pregnancy.

Testing the Water

Between 2014 and 2016, the team set out to discover whether their suspicions were correct. The mares and foals in both the Pennsylvania and New York farms were given physical examinations and had blood samples taken frequently. Their food, water and grazing soil were also tested for known toxic chemicals such as heavy metals and mycotoxins recurrently. Continuous passive sampling of the air and water was also carried out for PAHs. This is done by a collecting device constantly gathering samples of the medium it is placed in and testing for a specific compound. Lastly, the foals were evaluated for whether or not they had dysphagia.

Discovering the Link

Throughout the study, 65 foals were born, 17 of which were dysphagic. All 17 of these horses were born on the farm in Pennsylvania. The likelihood of a foal being born with dysphagia increased for every month their mother resided there while pregnant but mares who spent the first half of their gestation in Pennsylvania then moved to New York never had a dysphagic foal. They also found that males were more likely to have the defect than females.

After analysing the water samples, the team found that concentrations of the PAHs 3,6-dimethylphenanthrene, fluoranthene, pyrene and triphenylene were higher in Pennsylvania than in New York. However, partway through the study, the Pennsylvania farm installed a water filtration and treatment system for the horses' drinking water. The outcome of this was the concentrations of PAHs became the same or lower than those in New York. Dr Mullen continued to observe new-borns in Pennsylvania for two more years and found none of the 29 foals born had dysphagia after water treatment was put in place.

In a subsequent study, Dr Mullen found that the athleticism of a horse, determined by various factors associated with their ability to race, was not negatively impacted by a dysphagic past.

What Does This Mean?

Dr Mullen's work has given a critical insight into how UNGD may impact the surrounding environment and the organisms living there. She suggests that foals born within proximity to fracking sites are more likely to exhibit dysphagia, likely due to altered neurological functions. Because foals did not show dysphagia when their mothers moved away from UNGD sites after their first trimester, Dr Mullen determined that the negative effects are more impactful during mid- to late-gestation.

As PAHs can cross the placental barrier and can also be found human breast milk, Dr Mullen believes her research with horses can serve as a model for humans. PAHs can cause DNA damage, perhaps leading to neurodevelopment problems in babies, just like in the foals.

Considering the potential health implications for humans as well as animals, Dr Mullen explains that continued research is important so that we can further understand how unconventional natural gas development may alter the lives of those who reside close by.

Meet the researcher

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Dr Kathleen Mullen completed all three of her degrees at Cornell University in Ithaca, New York. She holds a Bachelor of Science in Animal Science, a Master of Science in Agricultural Economics and a Doctorate in Veterinary Medicine. After working as an associate at a large animal ambulatory practice in Central Massachusetts, she returned to Cornell University where she held a residency and then an instructorship of Large Animal Internal Medicine. As well as teaching veterinary students at Cornell and in Haiti, Dr Mullen has presented and published her research in multiple veterinary journals. She now works at Littleton Equine Medical Center in Colorado, where she practises as a veterinarian in addition to her research.

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HOOKAH (I.E., WATERPIPE) SMOKING: UNDERSTANDING USER PERCEPTIONS AND HEALTH RISKS

Hookah smoking is the least regulated tobacco form. It is rapidly gaining in popularity to the extent that we are now facing a contemporary epidemic of tobacco abuse. Of particular concern is the level of usage among youth and young adults. **Professor Mary Rezk-Hanna** from the University of California, Los Angeles works with a group of scientists who aim to drive policy regulation of tobacco and alternative tobacco products, including hookah smoking, by investigating their health effects on the cardiovascular system.

History of Hookah Smoking

Hookah smoking, otherwise known as shisha or narghile, are all terms that describe the act of waterpipe smoking; a specific form of tobacco use. The hookah smoking mechanism begins with the passage of charcoal-heated air through perforated aluminium foil and across the tobacco, known as maasel. The smoke generated from this process bubbles through the water in the pipe and is then inhaled by the smoker.

Hookah smoking is a historically Middle Eastern and Indian form of tobacco use, previously regarded as a habit confined predominantly to older males and generally not taken up by many. However, the contemporary trend of this ancient practice is no longer limited to this societal demographic, but rather, has transformed into a public health crisis with particular concern being raised about the usage among both male and female youth and young adults.

The spread first reached the youth in the Middle East, and soon after, youth in North America and Europe. The change in demographic and popularity of hookah smoking observed in the USA in the 1990s has been attributed to; the availability of fruit- and candy-flavoured tobacco, advertising on social media, lack of tobacco and waterpipe design regulation, and unsubstantiated claims regarding its safety. Stylish hookah lounges and cafés have since been on the rise, with reports of increasing numbers, especially around USA college campuses.

Recent surveys report that 1 in 6 adults in the USA use hookahs to smoke tobacco, and young adults are the most regular users. Using USA nationally represented data from the Population Assessment of Tobacco and Health study, Professor Rezk-Hanna and her team recently showed that compared to heterosexual adults, sexual minority adults have higher rates of ever and current hookah use. The study also highlights the rapid uptake of electronic

nicotine products (e-products) in addition to hookah use because among sexual minority adult current hookah smokers, hookah plus e-product use increased by 97% from 2013 to 2014 (2014: 3%; 2014: 6%). Hookah smoking is as common as smoking cigarettes which calls for concern considering hookah can be used to smoke other products, historically opium or hashish, and thus hookah serves as a potential gateway to other products.

Professor Mary Rezk-Hanna at the University of California specialises in tobacco research and tobacco-related cardiovascular diseases. Her team focuses on investigating potential

‘We believe that this line of inquiry will lead to a better understanding of the human health effects of new and emerging tobacco products, and in doing so, help build a scientific basis to inform tobacco product regulation’.

problems of emerging tobacco products, especially hookah, and the short- and long- term implications of this on cardiovascular function; a term used to refer to the heart and blood vessels. She aims to uncover why society’s younger population struggle with hookah smoking, and ultimately use her research to improve the regulation of hookah and other nicotine delivery systems by challenging existing claims that it is a safer tobacco option.

Perceptions Around Hookah Smoking

A widespread belief exists that, unlike cigarette smoking, the tobacco from hookah smoking is harmless because it is filtered as it passes through water. This belief has fuelled its marketing as a safer, non-addictive alternative to cigarette smoking. Hookah is the only form of tobacco use that is not regulated in the USA and it is exempt from the clean indoor air legislation; an act prohibiting smoking in public places. Trendy hookah lounges and cafés designed specifically for social smoking, stylised hookah pipes and flavoured tobacco are becoming commonplace; encouraging this unhealthy practice. The younger population is the targeted consumer due to these marketing strategies and loose regulations, and this plays a key role in shaping attitudes towards hookah smoking in this demographic.

In 2014, Professor Rezk-Hanna and her colleagues studied young adult hookah smokers’ perceptions, attitudes, beliefs and preferences toward hookah smoking. They recruited hookah smokers aged between 18 and 30 from hookah lounges in Southern California and asked a sample of them to complete a survey about their perceptions and habits related to hookah.

It was found that almost three-quarters of the participants smoked more than once a week, and around a quarter of them smoked four times or more in a week. The survey indicated that just over half of participants believed that hookah was not harmful to their health, almost half believed that hookah tobacco gets filtered out through water, and around a third believed the fruit flavours have a detoxifying effect.

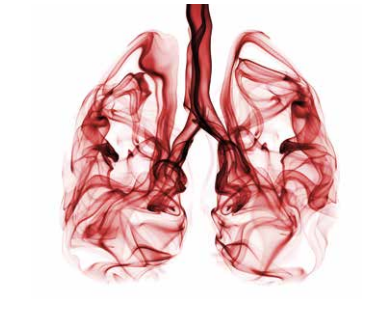
Professor Rezk-Hanna and her team concluded that hookah smokers shared similar perceptions and beliefs that hookah is less harmful and less addictive than cigarettes, with some believing this to be due to the absence of nicotine; a belief not grounded in evidence. The preference of smoking hookah over cigarettes was largely due to the social aspect (including shape making with smoke), and also the appeal of the fruity and candy flavours; a concept that is banned for cigarettes in certain countries. One long hookah smoking session was favourable to the participants compared to multiple single cigarettes per day, resulting in exposure to higher volumes of tobacco smoke.

The lack of knowledge regarding hookah’s harmful effects on health, alongside the continual glamourisation of the hookah smoking experience, is a growing concern for Professor Rezk-Hanna and her team. They believe raising public awareness on hookah smoking through community and public educational programmes, as well as enforcing stringent rules around its use is required.

Health Risks Associated with Hookah Smoking

Unlike cigarette tobacco, hookah tobacco does not remain lit independently; charcoal must be placed on top of the tobacco to maintain its continual burn throughout a smoking session. The charcoal briquettes used to heat the tobacco do not achieve temperatures as high as combustible cigarette smoking, therefore, hookah smoke contains higher levels of incomplete combusted products than combustible cigarette smoke, including higher carbon monoxide (CO) levels.

Professor Rezk-Hanna and her team of collaborators have evaluated the cardiovascular effects of hookah smoking in their research over the past several years. Using myocardial contrast echocardiography – a perfusion imaging tool that utilises tiny gas-filled microbubbles (~1-8 μm in diameter) that are destroyed with high intensity ultrasound to assess coronary blood flow by subsequently measuring the rate of microbubble replenishment – they found that among young healthy hookah smokers, similar to cigarette smoking, short-term hookah smoking causes an acute increase in coronary blood flow, presumably a physiological response of nicotine or other constituents found in hookah smoke.



In recently published research in 2019, Professor Rezk-Hanna and her team attempted to dissect out this centuries-old yet understudied complex tobacco product down into its key components. First, they studied the combined effect of hookah-flavoured tobacco heated traditionally with charcoal briquettes on endothelial function. This refers to disturbance in the lining of the arteries which can impair their functioning and is one of the earliest signs of developing cardiovascular disease. Then, they removed the effect of charcoal combustion by repeating their measures when the same hookah flavoured tobacco was heated electrically by e-charcoal and compared that to cigarette tobacco. Finally, they isolated a key charcoal combustion product that distinguishes hookah from cigarettes by comparing responses of smoking charcoal-heated hookah to those seen when the same hookah smokers inhaled a 0.1% CO gas mixture to match their CO levels achieved with hookah smoking.

Similar to cigarette smokers, hookah smokers had high concentrations of nicotine in their blood, which contributes to the constriction of blood vessels and limiting blood flow through them. However, expired levels of CO increased 9- to 10-fold in hookah smokers who smoked charcoal-heated hookah compared to those who smoked electrically-heated hookah or cigarettes, indicating that traditional hookah smokers had greater levels of CO in the body. Furthermore, while charcoal-heated hookah smoking increased endothelial function, smoking electrically-heated hookah tobacco or cigarette tobacco similarly decreased endothelial function. The production of high levels of CO from charcoal briquettes, which is known to dilate blood vessels, appears to mask the negative effects of charcoal-heated hookah tobacco smoke to impair vascular function.

Taken together, Professor Rezk-Hanna and her team provide evidence that hookah tobacco smoking, similar to cigarette tobacco smoking, appears to harm the ability of blood vessels to function, and the presence of incomplete combusted products such as CO are additional constituents that have further health implications, counter to social media claims that it is a harmless cigarette alternative.

The E-Hookah Device Gaining Popularity

Electronic nicotine delivery systems (ENDS) are rechargeable battery-operated devices that consist of a power source and a heating element. They are used to mimic conventional tobacco products but do not require combustion. Whilst e-cigarettes are the most widely used ENDS, e-hookah pens and bowls are gaining popularity, especially among younger members of society.

E-hookah vaping devices were introduced in 2014 – recently patented by the American tobacco manufacturing company Philip Morris – and marketed as a healthier, and possibly more convenient, alternative to traditional charcoal-heated hookah smoking. Little is known about the health risks associated with the different types of e-hookah vaping devices, meaning these marketing claims are not evidenced. While e-hookah pens are similar in design to e-cigarettes, e-hookah bowls are combined with and placed on traditional waterpipes, allowing the flavoured aerosol to pass through a water-filled base before being inhaled.

In research published in 2019, utilising the USA nationally representative data, Professor Rezk-Hanna and her team found that e-cigarettes and e-hookahs differed by product characteristics, with e-hookahs more commonly possessing candy flavours, whilst e-cigarettes were tobacco or menthol flavoured. The differing flavours have influenced perceptions of e-hookahs, with young people describing them as 'cool' and associating them with being up-to-date with new technology. This has resulted in a distinctly different user profile demographic between e-hookahs and e-cigarettes. Beyond flavour perceptions, there is no known research on the specific toxic constituents in the flavours or associated health risks of e-hookahs. A recent study conducted by the Rezk-Hanna laboratory showed that despite the absence of tobacco combustion and contrary to claims that the presence of water 'filters out toxins', flavoured e-hookah vaping acutely impairs the ability of blood vessels to function.

Looking to the Future

Professor Rezk-Hanna, along with other scientists, has helped inform the short-term cardiovascular implications of traditional charcoal-combusted hookah smoking. A large effort in her laboratory is now aimed to investigate the long-term cardiovascular consequences of hookah smoking among flavoured hookah tobacco smokers as compared to conventional cigarettes smokers and healthy non-smokers. Because of the recent remarkable uptake in vaping among youth, Professor Rezk-Hanna has extended her work to examine the health effects of e-hookah bowl vaping and the relative contributions of nicotine versus flavoured electronic aerosol in mediating the vascular effects associated with vaping.

The research of Professor Rezk-Hanna and her team has provided evidence that counters the social media claims that hookah smoking is a safer alternative to cigarette smoking. She is committed to using these findings to drive policy to regulate hookah, and ultimately, slow down this tobacco epidemic primarily affecting youth which has been completely overlooked by the popular press.

Meet the researcher

Professor Mary Rezk-Hanna
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Born and raised in Egypt, Professor Mary Rezk-Hanna obtained her PhD in cardiovascular physiology from the University of California, Los Angeles School of Nursing, where she currently serves as an Assistant Professor. As the director of the Integrative Cardiovascular Physiology Laboratory, her work focuses on investigating the cardiovascular effects and potential harms of conventional, new and emerging tobacco products and the mechanisms by which these products affect cardiovascular function. More specifically, Professor Rezk-Hanna is studying the short- and long-term changes in physiological function associated with tobacco products use, including hookah (i.e., waterpipe) smoking and the integrative (systemic to molecular) biological mechanisms that mediate these physiological changes. A recently added direction of interest involves examining the vascular effects of second-hand exposure to vaping aerosol, an understudied topic that led to constructing a smoking room, housed within the UCLA Center for the Health Sciences building, specifically designed for conducting acute smoking or vaping exposure studies in a controlled laboratory environment without exposing research personnel to smoke or aerosol. Her goal is to use physiologic data to inform science-based public policies for regulating tobacco products and electronic nicotine delivery systems to protect public health. Among a multitude of other awards, in 2020 she was appointed as Fellow of the American Heart Association, in addition to receiving the Marie Cowan Promising Early Career Investigator Award.

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WORKING TOWARDS SAFER TATTOOS

The potential health risks of tattooing are known. However, many of the regulations which govern the practice of tattooing are somewhat relaxed compared to other industries. **Dr Christopher Hohl** and the Chromatography Section at the State Laboratory Basel-City, Switzerland, work to analyse the composition of tattoo inks and investigate the effects of tattooing to provide the authorities with the evidence needed to improve tattoo safety standards.

Tattoo Inks and Consumer Safety

A notable minority of the population is tattooed. Many of the health risks associated with tattooing are published. What is perhaps surprising, however, are the relatively few safety regulations which govern the sale and application of tattoo inks, compared to many other consumer products. The Chromatography Section at the State Laboratory Basel-City, Switzerland, is working to identify harmful substances associated with tattooing. One of the section's goals is to gather evidence to help regulatory bodies enhance the current tattoo safety regulations and protocols.

Tattoo ink consists mainly of one half a mixture of individual pigments, and the other half solvents with dispersing agents. Each pigment is chemically diverse in structure and responsible for a specific colour. The pigments absorb specific frequencies of white light and reflect all others for the human eye to perceive. The frequencies of light absorbed are dependent on the precise chemical structure of the pigment.

The overall colour of an ink largely depends on the type of and relative quantity of each pigment present. Different shades of ink colours are achieved by adding either 'carbon-black' (which resembles soot) for darker shades, or titanium dioxide for lighter colours. Pigments are only sparingly soluble in solvents, such as water, alcohol and glycerine. Consequently, dispersing agents are added to help produce a more uniform mixture which can be easily injected under the skin.

From a consumer safety standpoint, the main issue with pigments used for tattoos is the complete absence of relevant tests in this respect. The reason for this deficiency lies in the origin of the pigments, which were not produced for usage in tattoos. Manufacturers developed them to be used in automotive, paper and plastic paints, for example, which are predominantly tested for their stability when exposed to sunlight without consideration of human toxicity. The range of concoctions available on the market presents a significant challenge to the Chromatography Section, who invest much of their time unravelling the composition of imported products. Ultimately, their research will help guide authorities on possibly banning the import and usage of harmful pigments.

Systematically Detecting Harmful Substances

There are several technical challenges which the Chromatography Section must overcome. Since the pigments are only sparingly soluble in common solvents, it is quite difficult to prepare analytical samples with detectable concentrations of the pigments. The background signals produced by many analytical instruments overlap and interfere with the signals produced by the sample. The section addresses this issue depending on measurement technique, by first removing much of the accompanying materials, including the solvent, prior to analysing pigments in the cleaned-up particulate matter. In other cases, the pigments are (partly) dissolved using special solvents.

Once the samples are ready, the Chromatography Section employs a range of analytical techniques which, in many ways, produce patterns of signals or fingerprint-like profiles which are characteristic of specific components of a mixture. By comparing the profile with known substances, the section can quickly identify whether a substance is present.

The main technique that the Chromatography Section utilises is called mass spectrometry (MS). The pigment samples are passed into the instrument, which then breaks down most of the sample into smaller fragments and fires everything towards a detector. When a pure, single pigment is analysed the signals are comparatively simple to interpret because they only represent the sample and its fragments. The collection of signals makes up the identifiable 'fingerprint'. However, in relation to a mixture of components, as the section's research entails, MS produces signals which overlap and are therefore difficult to assign. It becomes more difficult to determine the origin of each signal. This is very much like trying to identify one human fingerprint which has been overlapped by several other fingerprints.

Prohibited Pigments and Suspect Labelling

The specific type of MS that the Chromatography Section applies to pigments is known as 'time-of-flight MS'. Every ink sample is made up of compounds with a different mass. Inside the instrument, the sample and its fragments are fired from the same starting position with the same kinetic energy towards a detector. The kinetic energy of a particle in the instrument is proportional to its mass and proportional to the square of its velocity. If all particles have the same kinetic energy then lower masses will migrate with greater velocities and vice versa. As a result, each particle is separated along a linear beam according to its mass: lighter masses collide with the detector before heavier masses, hence the term 'time-of-flight'. Subsequent calculations then reveal the relative mass and probable identity of each particle.

The experimental approach is only qualitative, that is, it only shows which pigment was present in the sample. Time-of-flight MS does not indicate how much of each pigment was present in the ink sample.

In the literature, pigments are sometimes symbolised by a code, for example, 'Pigment Yellow 14' where the number identifies the specific pigment compound. In 2016, the Chromatography Section published their time-of-flight MS analyses of inks in use at the time. In samples gathered during market surveys between 2009 and 2017, the Section discovered that the prohibited 'Pigment Green 7' was the second most common pigment present! About 7% of legal pigments were not declared on the product labels while more worryingly, about 68% of illegal pigments were not declared. Some green inks which were labelled as mixtures of yellow and blue pigments which would explain the green colour, containing 'Pigment Green 7' which is banned in Switzerland. By analogy, several magenta inks, labelled as mixtures of red and blue inks, were found to contain illegal 'Pigment Violet 19'. The section concluded that their findings were indicative of label forgery taking place somewhere along the supply line, highlighting the urgent need to review marketing and import procedures.

From left to right first row: Theresa Otz, Sandra Lang. Second row: Franz Dussy, Markus Niederer, Thomas Stebler, Urs Hauri, Urs Schlegel, Christopher Hohl. Absent: Nadja Ryser, Cornelia Hamberg.

Going Deeper, Under the Skin

Another theme of the research by the Chromatography Section looks at the fate of tattoo pigments after injection under the skin. Pigments are known to fade over time and this is partly due to the light-induced decomposition of tattoo pigment molecules. The energy of light can cause a pigment's chemical bonds to break, changing the colour of the affected site. Other possible reasons for fading include the transport of the pigment molecules from the skin's dermal layer to other parts of the body, as well as the chemical breakdown catalysed by nearby enzymes. In fact, the light-induced tattoo removal process by laser treatment follows very similar principles. Laser treatment simply accelerates the breakdown of pigments.

The fate of both legal and illegal pigments in the dermal layer has only sparsely been studied. Dr Hohl explains, however, that light-induced degradation of pigments can yield products, some of which are potentially cancer causing. He also argues that while most pigment molecules are almost water insoluble and may not present a health risk, this does not hold true for light exposed tattoo pigments located in the dermal layer.

Many compounds with a characteristic nitrogen-nitrogen double bond, of which many pigments are examples, break down to yield compounds which are potentially cancer causing. A commentary by the section revealed how much of a known carcinogen, derived from light exposed 'Pigment Yellow 14', is released over time. The section then extrapolated the trend to predict carcinogenic levels over several years. Overall, they estimated that 'Pigment Yellow 14' present over a tattooed area of about 400 cm^2 adds about 5 extra cancer cases per 10,000 people. By comparison, smokers add about 420 extra cancer cases per 10,000 people. In the USA, one extra case per one million people is tolerable. Their report concluded that much work is needed to reduce the number of new tattoo related cases.

Fading Tattoos

The Chromatography Section set about modelling the effect of ultraviolet and visible light on tattoo pigments under the skin. Given the limited water solubility, pigments often exist as tiny crystals within the dermal layer. The section mixed known ink mixtures as a suspension in water, sandwiching the sample between two glass plates and then irradiating the plates with light. Not all pigments decomposed appreciably. The section found that for the cases where decomposition did occur, the process involved the breaking of the nitrogen-nitrogen double bond. Interestingly, the section found that the degradation of ink mixtures was generally faster than the degradation of pure pigments. They found that titanium dioxide sped up the light-induced breakdown of 'Pigment Yellow 14'. These results show that pigment degradation as part of an ink mixture is quite different to the more simplistic model which studies pure pigments as samples.

The Chromatography Section also outlined a few limitations with their model, some of which represent areas of further study. They noted that they did not simulate the transport of pigments, or their breakdown products, from the site of injection. Consequently, the pigment components and their breakdown products would persist at their place of origin. This complicates matters because the residual compounds decompose on further irradiation or react further, yielding yet more products, when they would not normally do so in a human host.

Currently, safety standards in the tattoo industry are lacking. The vital work of the Chromatography Section will enable the authorities to review and raise the standards, and most importantly, protect the consumer.

Meet the researcher

Dr Christopher Hohl
Chromatography Section
State Laboratory of Basel-City
Switzerland

The Chromatography Section headed by Dr Christopher Hohl is part of the State Laboratory of Basel-City in Switzerland. It is an official authority responsible for the enforcement of federal laws concerning, among others, the consumer safety of food and utensils of daily use. The section consists of Dr Hohl and three groups, each with a PhD chemist and two laboratory technicians. In their work, market surveys using chemical analysis are an essential tool for checking the legal conformity of products taken from the market. Chromatographic techniques used by the section include gas chromatography, high performance liquid chromatography, and high performance thin layer chromatography. In addition, time of flight mass spectrometry also plays an important role. The section is focussed on determining the organic compounds of health concern in cosmetics, toys, tattoo inks and e-liquids, including also biotoxins in food and beverage, as well as detecting fraudulent colouring of seafood. Confronted with a wide variety of problems, where analytical methods either do not exist or lack performance requirements, in-house method development is of utmost importance. The qualified and well-established section not only develops and employs innovative methods fit for purpose but uncovers previously unknown safety-relevant problems. One of these previously underestimated problems is the photodegradation of certain tattoo pigments.

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EVIDENCE-INFORMED DECISION MAKING: BREAKING DOWN THE WALLS BETWEEN RESEARCHERS AND POLICY MAKERS

Anyone who has worked closely with government, be it local, national or international, will know the frustrations of trying to influence policy and decision makers. The 'dark arts' of the political analyst and the political lobbyist, particularly those that seem to succeed in having influence, remain a mystery to many. **Dr Logan M. Lawrence** at Dalhousie University, Nova Scotia, explores the concept of 'policy capacity' to try and understand, measure and operationalise the best approaches to assist and support policy makers to make 'the right decisions'.

Health Policy: Research into Practice

Like many working in health policy, particularly those in preventive or public health, Dr Logan M. Lawrence at Dalhousie University, Nova Scotia, understands that there is an enormous chasm between the production of academic, evidence-based research, and the implementation of the findings from that work to become adopted policy in the 'real world'. He acknowledges that early in his training, like many, he held the common misconception that to influence policy makers, we must simply provide them with the 'right' information. It can be tempting to believe that if only academics could better present and translate their findings, then decision makers would introduce the logical improvements that have been recommended.

The field of public health presents significant challenges. The determinants of health and the prevention of ill-health are multi-layered and complex, and next to impossible to summarise succinctly. Traditional health prevention interventions, such as tobacco control and physical activity, focused primarily on individual behaviours, can take decades to produce health gain in the population and may come at a high economic cost or societal change. More recent topics, such as health equity, acknowledge the roles of structures and systems which contribute to health, but changing these systems requires more than making a case for their flaws. Finally, health policy can be highly politicised, making it even more challenging to interact in the political world of policy makers.

When preventative public health is competing for scarce health resources, it is often easier for policy makers to grasp the immediate benefits and short-term outcomes of hospital and secondary care. Policies of more doctors and nurses, cheaper drugs and more specialist equipment are quick headline grabbers, and can appease the strong and powerful lobbying from the medical professions, even when the evidence on long-term effects and cost savings favour other options.

Dr Lawrence studies the complex factors that influence policy decision making through two synergistic approaches. The first is through knowledge translation and evidence-informed decision making; understanding how knowledge is created and shared to support decision making processes. The second is through studying 'policy capacity', which in its simplest form, refers to the ingredients which help policies succeed. Underlying the concept is a focus on identifying the individual, organisational, and system-level abilities required to best inform and implement policy decisions. With the combination of these two areas, Dr Lawrence hopes to better understand and assist the passage of research into policy and practice.

Policy Capacity

Dr Lawrence and colleagues examined studies of policy capacity over the past two decades. Through this, they identified a broad range of definitions. The concept of policy capacity was generally described in one of three ways. The first was related to improving the likelihood of helping policy makers arrive at a successful policy decision or solution. The second considered the need to adapt existing policies to improve societal outcomes even in the face of challenges. The third description was related to policy capacity being a tool to measure government performance.

Dr Lawrence and his colleagues identified a conceptual framework developed by researchers in 2015, which provided a 'comprehensive and multifaceted conceptualisation of policy capacity'. The framework consisted of nine interrelated elements (sub-capacities) that reflect the components of policy capacity at a resource level (i.e., individual, organisational and system levels) and through the competency areas of those involved (i.e., analytical, managerial/operational and political). However, the team identified gaps in the framework's operability, nothing that 'further work was required to determine its usefulness in assessing policy capacity across policy areas, particularly as a practical tool for policy makers and scholars to assess and compare policy capacity in different cases'.

The Dalhousie University team then developed a tool for systematically and transparently assessing policy capacity. By drawing on the input of health policy experts in academia and the health system, they generated 40 factors and 131 indicators for assessing the nine sub-capacities. The adapted tool could, for the first time, be used for assessing policy capacity across several resource levels and competency areas by those working close to government, to measure their ability to produce successful policies. Dr Lawrence and his colleagues believe the tool will be improved through use by experienced policy practitioners, and state that 'users are encouraged to use their judgement to determine which items are most relevant for their purposes, and [to] identify and test new factors and indicators that suit their needs and context'.

Dr Lawrence and colleagues hope that future testing of the tool, across a variety of policy areas and changing the weighting or prioritisation of different elements, will enable the tool to 'craft a narrative around policy capacity … (i.e., whether indicators denote factors, which denote sub-capacities, which denotes policy capacity, which leads to policy success)'. The team hopes that knowledge and insights obtained from the tool will provide direction for future efforts requiring a large degree of policy capacity.

Integrated Knowledge Translation

A second strand of work has been undertaken by Dr Lawrence and his colleagues to understand how knowledge is created and shared to support decision-making processes.

Policy makers working in complex fields such as public health are often faced with 'wicked problems': multi-dimensional and dynamic issues which can cross social and political contexts, and where positive effects need to be balanced against simultaneous negative consequences. For example, to fund a new service may require the decommissioning of an existing service, with all the human and social costs that may entail. Yet wicked problems have no single solution, so the creation of a new service at the cost of an old one will be insufficient to resolve the problem. Rather, multiple strategies need to be pursued, collectively adding to the possibility that the problem will be meaningfully diminished.

To formulate and justify their recommendations, public health practitioners rely heavily on evidence-

based decision making, which has been defined as the synthesis, interpretation, translation and utilisation of large quantities of evidence in order to arrive at the best possible solutions. A perceived flaw in evidence-based practice is perhaps that it can be perceived as 'expert driven' and not always reflective of political realities; a factor which may deter policy makers. Therefore, Dr Lawrence and colleagues have investigated the concept of integrated knowledge translation (iKT), as a mechanism to engage users (e.g., clinicians and patients, but particularly policy makers, the intended recipients of the research evidence), as active participants in the research process. These participants are known as 'knowledge users' (KUs).

For the approach to generate meaningful research that can be used by KUs, the KUs need to be included in the research process from the very beginning; establishing and refining research questions, developing research methods and participating in analysis and dissemination of results. Equally, researchers will be encouraged to think about how their research can be conducted in order to maximise its benefit to others in society. It is often challenging for academics and specialists to work with people who may have no scientific training, but iKT is a process that facilitates these relationships to be built to achieve a common goal.

The idea of engaging KUs to help shape research has its roots in participatory action research. Participatory approaches are often used to create a sense of 'community ownership' and to build partnerships when attempting to deliver social change. The process has been shown to add value to research projects and increases engagement and sustainability of new projects, with the notable benefit of improving community capacity. iKT has gained popularity in Canada for its potential to co-create research-based solutions with those that will have ultimate authority in the decision-making process, but research conducted by Dr Lawrence and his colleagues notes a lack of meaningful engagement which is the hallmark of participatory research. The conditions required for effective iKT with policy and decision makers include flexibility of contributions and building longstanding relationships that extend beyond one-off research projects.

iKT in Practice

To explore how researchers and policy makers can work better together, Dr Lawrence was awarded a Health System Impact Fellowship from the Canadian Institutes of Health Research (CIHR) to work with the Government of Nova Scotia. The aim of this project was for Dr Lawrence to bring his research ability to support the development of new policies to improve the population's access to primary health care (PHC) while also giving policy makers an idea of how researchers can support their work.

As a frontline healthcare service, PHC is responsible for routine community based medical care and disease prevention and management, usually delivered by a general practitioner or 'family doctor'. The Nova Scotian government has committed to improving access to local health care providers, and particularly interprofessional 'collaborative family practice teams'. However, the reorganisation of existing complex services needs to account for differences in population need, while balancing patient preferences, evidence-based options and financial constraints.

Above and beyond his current work at the interface with local government, Dr Lawrence has expressed his general belief that knowledge translation also has a greater role in beginning to address the public's growing distrust in experts and science in the new 'age of ignorance' and fake news. He states that knowledge translation 'is a plan for getting your research out into the world'. Beyond publication in academic journals, he encourages researchers to look for wider opportunities for sharing their research with non-scientists, through blogs, podcasts, articles and discussion forums like Science cafes and TED talks, to open up awareness and conversations with a broader audience.

Through early and meaningful collaboration with others that have access to different social groups across different settings and contexts, such as policy makers, editors, journalists, comedians, graphic designers and celebrities, the research community can break down barriers between 'the experts' and general public, and encourage a wider understanding and appreciation of both the complexity and nuance of research and its relevance in everyone's daily lives.

Meet the researcher

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Dr Logan M. Lawrence completed a Bachelor of Science in Kinesiology at the University of Alberta in Canada, in 2012. He then completed a Master of Science in Kinesiology, at Dalhousie University, graduating in 2015. In 2020, he was awarded a doctorate in Health at the same university. Dr Lawrence has an overarching commitment to improving health policies and systems, working at the interface of health services and policy research, knowledge translation, and political science.

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FUNDING

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DISORDERS OF NUTRITION

TACKLING THE CAUSES AND CONSEQUENCES OF POOR NUTRITION

The second section of this issue showcases the vital work of researchers aiming to overcome the causes and consequences of poor nutrition. According to the Food and Agriculture Organization of the United Nations, between 720 and 811 million people across the world faced hunger in 2020. Most commonly found in the developing world, this form of malnutrition comes with a myriad of significant health consequences including stunted growth and increased risk of premature death. At the opposite end of the nutrition spectrum, the World Health Organization reports that in the developed world, obesity is at epidemic proportions, with health impacts including serious, chronic ill-health as well as increased risk of premature death.

We open this section by introducing the work of the Emergency Nutrition Network Wasting and Stunting Technical Interest Group (WaSt TIG). The WaSt TIG has been challenging the traditional perspective that children being wasted (thinner than they should be) and children being stunted (shorter than they should be) are two different outcomes of undernutrition with different causes and requiring different interventions. We read of their vital research indicating the need for a unified approach to address these nutritional disorders.

Conversely, being overweight is a well-recognised risk factor for the development of chronic diseases such as diabetes, heart diseases and certain types of cancer. We turn to the work of Dr Jamie I. Baum at the University of Arkansas, who is exploring the relationship between dietary protein intake and its impact on body composition and metabolism. We read how Dr Baum is working to develop efficient nutritional guidelines aiming to prevent and treat obesity.

The prevalence of obesity in developed countries such as the USA is a particular concern, exacerbated by data pointing to significant increases in the incidence of obesity over the past few decades. Dr Sadguna Anasuri at Alabama A&M University is exploring the possible impact of mass media on obesity in young adults. We read how her work has provided valuable insights into the dietary choices and fitness habits of young people, and how these insights could be used to inform effective interventions and public health policies.

Dr Michael Griffin at Sam Houston State University is exploring the role of transcription factors in obesity. Transcription factors are proteins that help turn specific genes 'on' or 'off' and Dr Griffin focuses on how early B-cell factor 1 (EBF1) assists in the formation of fat cells and tissue inflammation caused by obesity. We read how this inflammation may be the underlying cause of a multitude of diseases associated with obesity and presents an important treatment target for future drug design.

Pancreatic cancer is one of the devasting outcomes that can result from obesity but sadly, preventative measures and effective treatments are scarce. Dr Stephen Pandol at the Cedars-Sinai Medical Center in Los Angeles carries out broad and far-reaching research ranging from how lifestyle factors impact pancreatic disease to identifying the molecular and cellular mechanisms behind pancreatic cancer. We read how his dedicated work is driving forward the potential for better patient outcomes in this field.

We conclude this section by considering the potential role of diet and nutrition in the development of cancer, one of the leading causes of death worldwide. Dr T. Colin Campbell, based at Cornell University, proposes that cancer is not primarily a genetic disease as often thought but a nutrition-responsive disease. We read how Dr Campbell is providing convincing evidence that the consumption of animal-based protein plays an important role in the development of cancer and how a whole food nutritional approach could present an effective strategy for cancer prevention and treatment.

THE WASTING AND STUNTING TECHNICAL INTEREST GROUP: GENERATING EVIDENCE TO CHALLENGE THE DIVIDE IN NUTRITION

Despite improvements in children's nutrition over the past few decades, undernutrition remains a huge threat to the health and life of infants and young children worldwide. Health and nutrition actors have usually approached the problems of children being wasted, (thinner than they should be) and children who are stunted (shorter than they should be) as different outcomes of undernutrition with different causes and different interventions. Facilitated by the Emergency Nutrition Network (ENN), since 2014 the Wasting and Stunting Technical Interest Group (WaSt TIG) has challenged this view, and has begun to work to provide evidence for a unified approach to tackling these two outcomes of undernutrition.

Undernutrition: A Global Problem

Undernutrition occurs when a child consumes insufficient energy and nutrients to meet its needs for growth and to maintain good health. Insufficient intake may be due to a lack of food, a lack of appetite as a result of infection, increased nutritional needs due to infection or a combination of these factors. Undernourished children are found most often in places where hunger and disease are common. According to recent estimates by the United Nations Children's Fund (UNICEF), the World Health Organization (WHO) and the World Bank, 47 million children under 5 years of age are wasted and 144 million children are stunted. Undernutrition can have life-threatening consequences. According to the WHO, an estimated 45% of the deaths of children below five years old are associated with undernutrition.

The World Health Assembly Global Nutrition Target and the United Nations' Sustainable Development Goal (SDG) 2.2 is to reach internationally agreed goals on the number of stunted or wasted children under 5 years of age by 2025, and to end all forms of malnutrition by 2030. Efforts to meet these goals require a clear understanding of undernutrition, including the relationship between wasting and stunting.

Examining Wasting and Stunting More Closely

Wasting and stunting are often viewed as different conditions. This has led to different policies, programmes, research and financing mechanisms to tackle them separately. During emergencies, the focus of programmes has tended to be short-term, to treat wasted children and prevent deaths, while in longer-term development programmes the focus has been on preventing stunting and micronutrient deficits caused by long-term undernutrition. This is despite the fact that there are stunted children in countries experiencing a humanitarian crisis and there are wasted children in non-emergency contexts and in countries in which long-term development is the priority.

Credit: United Nations Children's Emergency Fund. Photographer: Mehedi Rahman.

‘The narrative around wasting and stunting … has generated a lot of discussion and reflection on how we are addressing wasting currently.’ (UN representative)

Credit: United Nations Children’s Emergency Fund. Photographer: Jacob Maentz.

This separation is despite the fact that as far back as 1973, the physiologist and child malnutrition expert John Waterlow reported on the links between the two, writing that ‘in practice, in a great many undernourished children, both processes will be at work’. Children can become wasted in weeks or even days because body weight can be lost quickly, while it often takes months for children to become stunted because a failure to grow in height is a slower process and in contrast to weight, height cannot be lost. Being wasted or stunted are the effects of undernutrition on children’s physical growth and provide measurable evidence that children’s growth/height and/or weight gain has faltered in comparison with well-nourished children. Children’s growth is impaired by these two types of undernutrition, which usually have the same causes – disease and poor diet.

To challenge this separation, in 2014 ENN established a WaSt TIG made up of volunteer expert researchers, and health and nutrition programmers and donors. The WaSt TIG began by examining the existing evidence on the relationship between these two outcomes of undernutrition, identifying and prioritising gaps in evidence, and then set about to fill some of those knowledge gaps.

Providing Evidence for the Connection Between Wasting and Stunting

One of the main achievements of the WaSt TIG has been the contribution made to strengthening the understanding of the relationship between wasting and stunting.

The work of the WaSt TIG has highlighted the growing evidence that a wasted child is at higher risk of becoming stunted while a stunted child is at higher risk of becoming wasted than a normal child. During the process of becoming wasted, a child’s growth in height slows until their weight has recovered, which identifies the role that preventing or treating wasting may play in preventing stunting.

Evidence collected by the WaSt TIG and other researchers indicates that 20–30% of children are born either stunted or wasted, a condition that already starts to develop in the mother’s uterus. Common risk factors for becoming stunted or wasted during infancy and early childhood have been identified but timing, intensity and combination of risk factors may lead to different outcomes in terms of weight and height. Some severely affected children will become both wasted and stunted.

Concurrent Wasting and Stunting: Who is Affected and What Are the Implications?

The WaSt TIG undertook an analysis to understand the prevalence, burden and outcomes for children wasted and stunted at the same time as this had never been reported previously. The analysis of data on children aged 6–59 months from 84 countries estimated that six million were concurrently wasted and stunted. Such children were found to be about 12 times more likely to die than non-wasted or stunted children, and were as likely to die as severely wasted children. Furthermore, the WaSt TIG found that once recovered from being wasted, these children appeared to be at greater risk for having subsequent periods of being classified as wasted.

Another important finding was that boys are significantly more likely than girls to be concurrently wasted and stunted, which is consistent with the higher prevalence of these conditions in boys when each outcome of undernutrition is examined separately. This greater vulnerability of boys to undernutrition, although previously acknowledged, has not been stated in nutrition policies and the implications typically have not been addressed by programmes.

Given this high risk of death for children who are concurrently wasted and stunted, the WaSt TIG has begun to identify ways of identifying and treating these children within current treatment programmes. It was found that two commonly used ways of measuring child growth – measuring either weight-for-age or mid upper-arm circumference – can identify these high-risk children. An urgent focus is now needed to define what type of care and treatment these

Credit: World Food Programme. Photographer: Mohamed Awadh.

Credit: World Food Programme.

children need to reduce their risk of death or of becoming even more undernourished.

Preventing Wasting and Stunting

Given the evidence that wasting and stunting share common risk factors, and that experiencing one of these forms of malnutrition leaves a child more likely to experience the other, the WaSt TIG has argued that having different and separate approaches to prevent children from becoming wasted and stunted is not rational. Instead, coherent and comprehensive measures to tackle the underlying causes of undernutrition are needed, including approaches that support mothers as well as children. Evidence suggests that the focus needs to shift from supporting children who are already wasted or stunted to children at risk of experiencing wasting and stunting, thereby interrupting as early in life as possible the processes that lead to a child becoming wasted or stunted. To do so, mechanisms to best identify and measure risk of undernutrition are needed in order to successfully prevent children from becoming wasted and/or stunted.

The Wasting and Stunting (WaSt) TIG

The WaSt TIG reviews its progress by coming together every two years to discuss emerging themes, priorities and the policy and programme implications of the research undertaken. A variety of outputs, including peer-reviewed academic papers, technical briefs, reports and blog posts have been published by members of the WaSt TIG, culminating in a Viewpoint article in the journal 'Lancet Child and Adolescent Health' in 2019.

A recent evaluation of WaSt TIG using a 'Story of Change' method found that successes have been driven by the way in which the WaSt TIG operates: it is made up of a mix of expert individual members who represent themselves rather than their institution's agenda and who function in an engaged, iterative, exploratory and task-orientated manner. The varied experience of the group's members, with expertise related to both wasting and stunting, the flexibility to allow individuals to set their own degree of engagement based on other commitments, and its open and collaborative structure, were also identified as key features of success.

Calling for Action

Since its inception in 2014, the WaSt TIG has identified evidence of a relationship between the processes of wasting and stunting, and the consequences of this for infants and children. The group has increased awareness among donors and development agencies of the limitations that dealing with wasting and stunting as separate problems places on their work, leading to critical shifts in thinking. As one respondent noted in the Story of Change evaluation, 'Everything we know about (the relationship between) wasting and stunting' is a product of this group.

A lot has been achieved by the WaSt TIG in a relatively short period and with limited financial resources. It has utilised existing data as much as possible and relied on open collaborations and data sharing. Both the interest and motivation of the people involved are very high and many give their time unpaid. Bringing together researchers with policy developers and nutrition programme staff has enabled discussions about how research can be used to improve our understanding of undernutrition, its consequences for children's growth, and how to prevent it.

The WaSt TIG has also identified important gaps in knowledge that have the potential to strengthen efforts to tackle undernutrition in childhood. This includes a better understanding of risk factors for undernutrition, how to identify children that are most vulnerable to becoming wasted or stunted (including identifying adolescent and pregnant mothers at risk of giving birth to undernourished children), and how to care for such high-risk children.

The opportunities and solutions to prevent both outcomes of undernutrition are the same: children need to be protected from disease, given a nutritious diet and grow to their potential, which requires integrated policies, programmes and interventions.

The WaSt TIG is calling for faster and more concentrated efforts to improve child health and reduce child mortality across the globe by approaching wasting and stunting together, as two intrinsically linked outcomes of undernutrition, particularly before children become wasted or stunted.

Meet the researchers

Wasting and Stunting Technical Interest Group

Emergency Nutrition Network

Oxford

UK

The Wasting and Stunting Technical Interest Group (WaSt TIG) is a group of experts in international nutrition that, since 2014, has worked to bring together existing and original research on wasting and stunting in children. The overarching aim of WaSt TIG is to better understand how wasting and stunting are linked, so that resources can be better directed towards improving health outcomes for children and reduce associated child mortality. WaSt TIG is coordinated by the Emergency Nutrition Network (ENN), a UK-based charity founded in 1996 and part of the international community of organisations and individuals seeking to reduce the global burden of undernutrition.

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We are especially grateful to Andrew Hall (of the WaSt TIG) for his support in developing this article.

THE BENEFITS OF A HIGH-PROTEIN DIET ACROSS THE LIFESPAN

Being overweight is a well-recognised risk factor for the development of chronic diseases such as diabetes, heart diseases, and certain types of cancer. As such, obesity represents a significant public health issue worldwide. It is the leading cause of death in the USA, notably in Arkansas, where **Dr Jamie I. Baum**, at the Department of Food Science at the University of Arkansas, is exploring, with her colleagues, the relationship between dietary protein intake and its impact on body composition and metabolism to develop efficient nutritional guidelines to prevent and treat obesity.

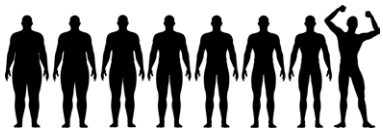
Obesity and Energy Expenditure

Obesity is a complex health issue resulting from a combination of different factors including genetics, behaviour, environmental and social influences. Although multifactorial, healthcare workers agree that obesity is often attributable to an imbalance between a person's daily energy intake and energy expenditure (EE). In other words, obesity results from too many calories being ingested versus the number of calories burned. The daily energy expenditure includes the resting metabolic rate (RMR), the energy spent during physical activity and the thermic effect of food (TEF). The RMR is the energy spent to cover our basic needs such as breathing and varies between each individual. The TEF is the energy required to digest, absorb and dispose of the nutrients and varies with the type of food ingested. For example, proteins (such as meat, fish and eggs) require more energy to be processed compared to carbohydrates (such as sweets and potatoes).

The Effect of a Protein-Rich Breakfast in Children

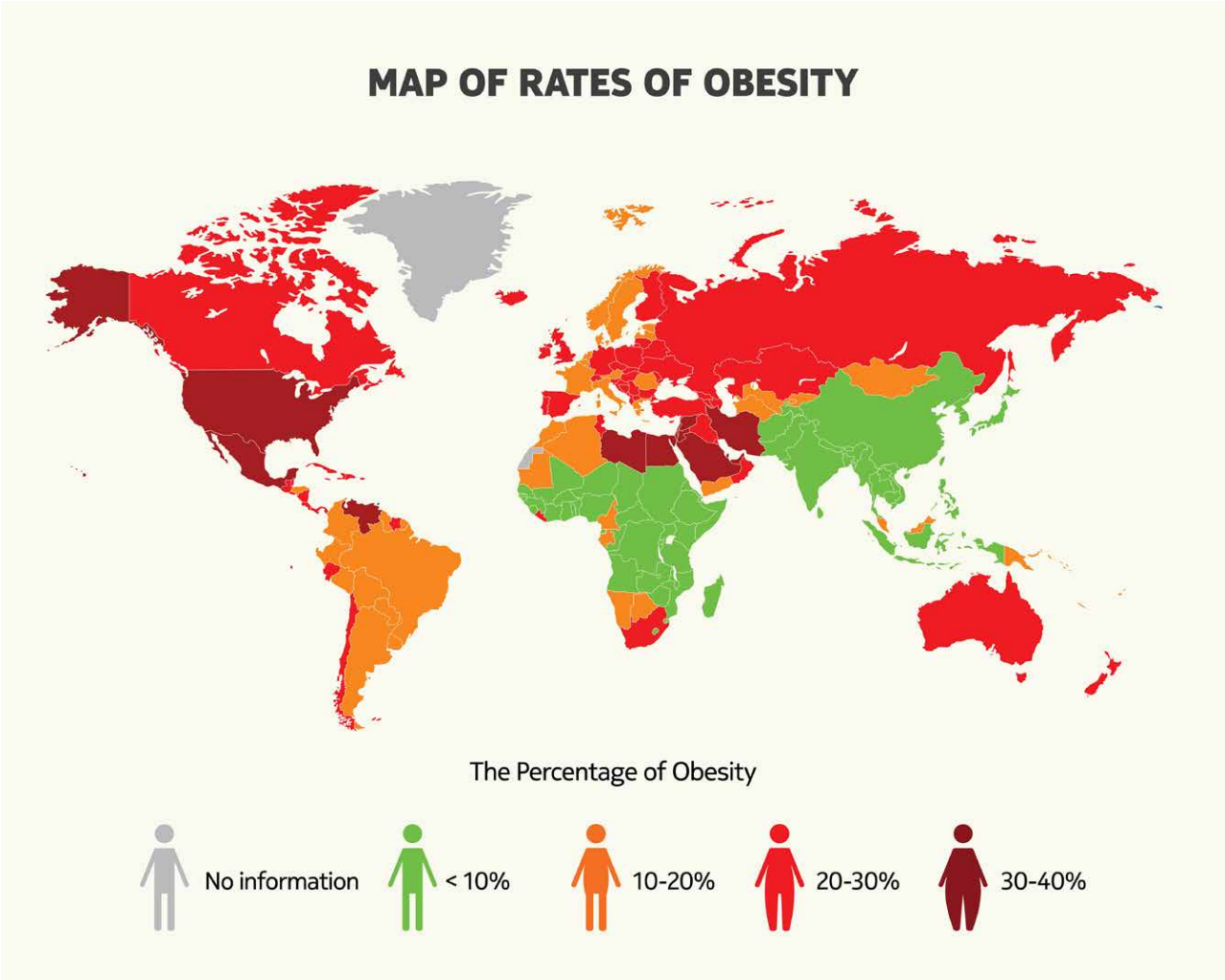
In the USA, about one-third of children aged between 2 and 19 years old is overweight or obese. The rise of obesity can be attributed to a lack of physical activity as well as poor nutritional education and habits. However, recent studies demonstrate that EE, RMR and TMF are also predictors of weight gain. Although this is well established in adults, there is a paucity of data in school-aged children. Dr Jamie I. Baum, at the University of Arkansas, is driving forward strategies to increase EE, RMR and TEF in normal-weight (NW) and overweight (OW) children as part of an effective, long-term, dietary intervention to tackle child obesity.

The challenge with childhood obesity comes from the necessity to built interventions easy to follow and effective in the long term. Dr Baum and her colleagues are observing the effects of increasing protein intake during breakfast in overweight and obese children. Their innovative study builds upon the simple idea that altering



macronutrient proportions in children's diet improves body composition, possibly through the regulation of metabolism and appetite. Using cutting-edge methods such as dual-energy x-ray absorptiometry (DEXA) and stable isotope methodology, Dr Baum's team can study changes in body composition (for example the proportion of fat and fat-free mass) and whole-body protein turnover. Protein turnover represents the balance between protein synthesis and protein degradation and is an important parameter of a healthy metabolism.

The preliminary results observed by Dr Baum and her colleagues are consistent with previous pilot studies conducted at the Center for Human Nutrition at the University of Arkansas, indicating that increasing protein intake



at breakfast had positive effects on NW and OW children by increasing TEF, fat oxidation and decreasing appetite. Furthermore, increased dietary protein intake at breakfast was also shown to increase the proportion of fat-free mass in NW and OW children after 6 weeks of intervention. This long term, scalable study has the potential to change children's breakfast habits, reduce and prevent obesity, and ultimately, change the dogma related to child nutrition.

The Primary Role of Fat-Free Mass in Regulating Adult Obesity

This is all the more important as new evidence demonstrates that fat-free mass plays a role in regulating the appetite, possibly by an 'energy-sensing mechanism' translating the energy requires by the fat-free mass into energy intake. The exact mechanisms about how the body's energy needs are sensed and translated into eating behaviour are still unknown, but a plausible explanation is that fat-free mass is associated with brain regions regulating appetite whereas fat mass is not. Skeletal muscle (muscles connected to the bones such as biceps, triceps, and so on) represents a large proportion of fat-free mass and plays a central metabolic role, notably in the whole-body protein turnover. They largely contribute to the RMR, which means the more skeletal muscles we have, the more energy we will burn at rest. Anterior studies have demonstrated that altered skeletal muscle metabolism is associated with increased risk of chronic diseases such as obesity, type 2 diabetes, and also cardiovascular diseases.

Protein synthesis is a biochemical process, which consumes ATP (adenosine triphosphate, the primary form of energy in the body) for each amino acid incorporated into the protein. The amount of muscle mass, therefore, determines the energy released for protein synthesis and the smallest mass change impacts the total energy expenditure.

Dr Baum and her colleagues are focusing on understanding the role of a protein-rich diet to increase skeletal muscle metabolism and increase the global energy expenditure in obese adults. Furthermore, it is thought that including specific amino acid also regulates appetite and self-selection of meals. For example, the branched-chain amino acids such as leucine, valine, and isoleucine stimulate muscle protein synthesis and promote transport of leucine to the brain. Leucine can be found in food such as meat, poultry, fish, and eggs, and is known to increase exercise performance, build muscle and

increase lean body mass. It has been shown in rodents that a leucine-rich diet reduces food intake and reduces body weight.

Dr Baum and her colleagues are collecting data to determine the effect of protein and branched-chain amino acids rich diets in obese and overweight adults to ultimately develop evidence-based nutritional recommendations to treat and/or prevent the development of obesity.

Increasing Protein Intake for Stronger Older Individuals

Sarcopenia is a condition characterised by the progressive loss of skeletal muscle mass and strength and is closely correlated with advanced age. The strong link between the length of lifespan and the loss of skeletal muscle mass and development of serious health issues such as obesity, diabetes and cardiovascular disease makes it critical to study how to improve skeletal muscle mass in the older population. Dr Baum addressed this critical issue in a review published in 2015.

Nutrition is an essential parameter of health and function in older adults. In her review, Dr Baum notes several studies highlighting the benefits of increased protein intake in maintaining a healthy energy balance, improving bone health and cardiovascular function. The current nutritional guidelines for protein intake, based on healthy college-aged men fail to address other health conditions such as injury, hospitalisation, surgery, or even simple ageing for which scientific studies recommend a higher protein intake.

Additionally, Dr Baum highlights that the quality of protein ingested is also a primary aspect for optimal health. As such, the protein characteristics, the food matrix in which the protein is ingested, and one's individual metabolism are three important aspects that are largely under-estimated in nutritional studies and associated guidelines. To illustrate this, several studies demonstrated, for example, that 'ingestion of milk proteins stimulates muscle protein synthesis to a greater extent after resistance exercise compared to ingestion of soy protein' and that animal proteins were significantly better preserving lean body mass than plant proteins.

There is an abundance of studies demonstrating the beneficial effects of a higher protein intake than the current recommendation. Dr Baum gathered scientific evidence to stress the need to revise the recommended dietary allowance of protein intake to achieve optimal protein utilisation. It remains of concern that older individuals suffering from chronic diseases may follow misleading recommendations and fail to ingest adequate protein levels.

New Nutritional Strategies Based on Solid Data

Together with her team, Dr Baum is implementing new strategies to prevent and treat obesity in children and adults. Although this is a well-studied field, current nutritional strategies are often inefficient or unsustainable. Dr Baum's research is based on the beneficial effects of higher protein intake at breakfast for children and focused on specific amino acid for adults. This simple, long term approach is backed up by sufficient scientific data demonstrating the beneficial impact of a rich protein and amino acid diet to increase skeletal muscle mass, energy expenditure and regulate appetite at all stages of life to achieve better health.

Meet the researcher

Dr Jamie I. Baum
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Dr Jamie I. Baum completed her BS in Dietetics at Urbana-Champaign, University of Illinois, where she later graduated in 2004 with a PhD in Nutritional Sciences. This was followed by a postdoctoral fellowship in Cellular and Molecular Physiology at Pennsylvania State University College of Medicine in Hershey. In 2007, Dr Baum relocated to the Netherlands where she was a research scientist both at Unilever Food & Health Research Institute and Danone Baby Nutrition. In 2011, Dr Baum moved back to the USA, to be part of the Department of Food Science at the University of Arkansas, where she is an Associate Professor with tenure. Dr Baum is also the Director of the Center for Human Nutrition. Dr Baum's research focuses on understanding the effects of a dietary protein on metabolism, body composition and energy balance in children, adults and older individuals to prevent the development of chronic conditions such as obesity and sarcopenia. Dr Baum's innovative and important work has resulted in her being the recipient of several awards and honours in the field of nutrition.

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Center for Human Nutrition

UofA DIVISION OF AGRICULTURE RESEARCH & EXTENSION University of Arkansas System

EXPLORING THE IMPACT OF MEDIA USAGE ON OBESITY AMONG YOUNG ADULTS

In the US, the number of children and adults with obesity or excess weight has increased exponentially over the past few decades, causing concerning effects on public health. Identifying factors that may play a role in this rise is of crucial importance, as they could help in devising effective strategies that promote healthier lifestyles. With this in mind, **Dr Sadguna Anasuri** at Alabama A&M University has carried out research exploring the possible impact of mass media on overweight and obesity in young adults, gathering valuable insights that could inform public health policies.

The Rise of Obesity

Obesity has developed into a significant health concern for many countries worldwide. In the US, the Centre for Disease Control and Prevention has reported a significant rise in the national obese and overweight population over the last few decades. In most US states and territories, over 30% of the population now falls into the obese category.

Excess weight or obesity can significantly increase a person's risk of developing several health conditions, such as diabetes, high blood pressure, heart disease, arthritis, stroke, fatty liver, renal disease, and certain types of cancer. Therefore, the rise of obesity in the US also means an increase in the prevalence of potentially life-threatening health conditions.

The Government and other national agencies have been trying to devise policies that could reduce the prevalence of obesity by addressing some of its root causes. However, this has proven to be quite challenging and ineffective so far. The recent rise in US obesity rates could result from several different factors, including higher consumption of fatty, salty, starchy, unhealthy foods, combined with an increasingly sedentary lifestyle. Although genetics, family history, certain medical issues, and some medications may lead to weight gain, the primary factor is often physical inactivity.

Dr Sadguna Anasuri has recently researched the impact of mass media on people's dietary choices and fitness habits. Through her work in this area, she aimed to recommend possible future interventions and policies that could help young people lead healthy lifestyles.

Impact of Mass Media

Although almost everyone now consumes a considerably large amount of media, young adults are spending an increasing amount of time online, using interactive social media sites such as Facebook, Twitter, Instagram, and YouTube. These are focal points where many food businesses, health companies, and fashion industries advertise their products. The advertisements that young adults are exposed to through TV and social media sites can have a significant impact on the products they buy and the habits they acquire.

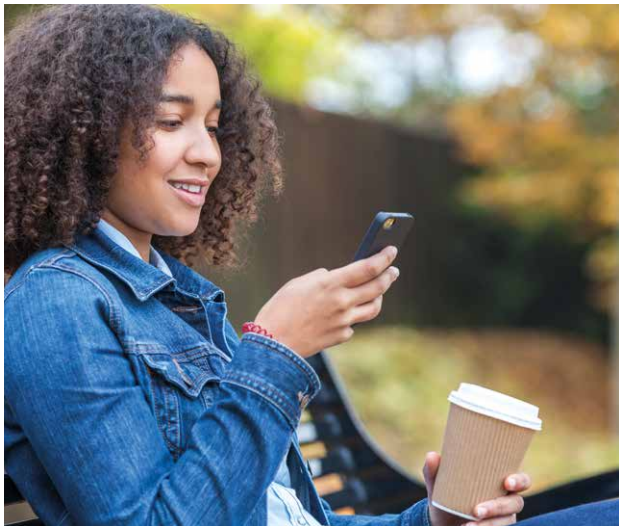
Dr Anasuri has been conducting extensive research investigating the impact of mass media on the food choices and health and fitness behaviours of young adults. In her recent work, she mainly focused on how

exposure to advertising or other forms of marketing on TV and online could play a critical role in the alarming rates of obesity, mainly in the southern state of Alabama.

Examining Previous Insights

As preparation for her exploratory work, Dr Anasuri reviewed previous research findings and theoretical models that could help in gaining a better understanding of the impact of mass media on the rise of obesity in the US and other countries. In a comprehensive review paper published in 2016, she utilised a conceptual model to illustrate the different factors influencing the dietary and media behaviours of young adults. This model delineates several factors, divided into three categories.

The first category includes ***personal factors***, such as cognitive-affective, biological elements, and individual behavioural patterns about food, eating preferences, and body-image. The second one outlines ***environmental***



factors, such as norms of the social or cultural group that the young person belongs to and their social environment (family unit, parenting practices, home environment). Finally, the model also focuses on the role of ***macrosystems***, including political or socio-economic systems at large, food availability, and mass media, and other individual factors such as lifestyle choices and nutritional status.

Dr Anasuri examined these different categories and presented the findings of past research that investigated the impact of various influencing factors on eating behaviours. Her conclusions showed that the advertising of unhealthy foods on mass media platforms is a major contributing factor to the increase in US obesity rates. The food, beverage, and restaurant industries' spending on digital media platforms is increasing rapidly – in 2019, it was $1.77 trillion. Also, it has been estimated that 11 out of 12 fast-food companies have Facebook, Twitter, and YouTube accounts.

Other research suggests that young people who watched TV programs containing food advertisements ate 45% more while watching, compared to those who did not. Another study found that children who had a TV in their bedroom were 1.3 times more likely to be overweight than those without one. Strikingly, one study found that the effects of being exposed to specific food advertisements can influence children's food choices even five years after they first watched an advertisement. According to research by Strasburger, Jordan, and Donnerstein, one of the reasons why mass media successfully influences children's behaviour could be their inability to understand persuasive intent and are thus highly influenced by advertising strategies. This highlights the need for parents to supervise their children's media usage, while also limiting the time they spend exposed to advertising.

Interestingly, Dr Anasuri's review highlights that many advertisements target youths from a cultural background, such as Latino or African American groups. Past research suggests that since 2003, advertisements for unhealthy foods and sugary beverages targeting African American children have increased significantly, far more than those targeting white children.

An Exploratory Study

One of Dr Anasuri's studies focused on media use and obesity among young adults between the ages of 18 and 26 years. It examined their exposure to advertising and health-related articles, participation in cooking and health groups, and whether they watched fitness videos available on social media platforms. The overall objective of her study was to identify the role that social media played on young people's food awareness, choices, and consumption, which could thus partly explain the recent growth in obesity rates.

She surveyed 2000 young adults with a 50-question survey that was administered both online and in printed form. It included questions about media use, perceptions of weight, eating habits, fitness practices, cooking at home versus eating out, online food shopping, family impact, and overall environmental factors that influenced their lifestyle. Dr Anasuri hypothesised that different forms of media – audio-visual, print, and web-based – would impact food consumption among young adults, lead to sedentary lifestyles, and eventually, weight gain. To test her hypothesis, she investigated young adults' media usage habits on their laptops, smartphones and other devices.

She also gathered a broad range of additional data related to young people's Internet usage, food and beverage choices, smoking and drinking habits, exposure to online literature on health, food and fitness, involvement in health and fitness groups, and attitudes towards product marketing. In addition to mass media, Dr Anasuri also focused on other social and cultural factors that could impact young people's consumption of unhealthy foods, causing them to gain excess weight. These factors included education, socio-economic status, family structure, and ethnicity.

Summary of the Findings

Among the demographic differences, gender had some influence on young adults' perceptions of body weight and their need to lose weight. Females ranked much higher on the frequency of their internet and phone browsing. Females also felt the need to exercise more at home and did more of their shopping online.

The participants' marital status showed a significant correlation with the frequency of their phone browsing, which included engaging with social media platforms. Surprisingly, income, ethnicity and parents' education did not seem to have any measurable influence on media consumption and Internet browsing. 'This shows that regardless of background, being a young adult was the most important factor when it came to media consumption,' says Dr Anasuri.

She then employed different statistical measures to investigate the significance of such connections. One measure she used was Multiple Factorial Analysis, which revealed that demographic factors mattered the least, food variables followed next, followed by environmental and fitness factors. The highest and most influential factor was their lifestyle, which included the usage of social media platforms, television, and the Internet at large. 'It was fascinating to note that fitness habits were highly correlated with media use and lifestyle factors,' says Dr Anasuri.

Potential Interventions

In her paper, Dr Anasuri also outlines some potential interventions aimed at decreasing obesity rates. Among different solutions, the first one involves starting early, by reducing the impact of unhealthy food advertisements on children's eating habits. This can be done by delivering public service announcements on mass media emphasising the importance of eating healthy, and parent-child communication aimed at teaching children not to be persuaded by advertising. Additionally, policymakers need to place restrictions on the amount of advertising for unhealthy food that is allowed during primetime when most families watch television.

The American Academy of Paediatrics has emphasised the importance of supervising children in their exposure to different forms of media, placing restrictions on the time they are allowed to watch TV or use smartphones and other devices. Some nutrition educators, along similar lines, have introduced initiatives aimed at using social media to increase physical activity through games and sports, and to promote healthier lifestyles and food choices.

Shaping Future Policies

The research carried out by Dr Anasuri provides valuable insights into how advertisements and marketing on mass media platforms could affect the lives of children and young adults. More specifically, it suggests that advertising unhealthy foods or beverages on social media platforms and TV greatly entices young people to buy and consume these products, increasing their chances of gaining weight.

'More research is needed to better understand how vulnerable teens and young adults are to digital marketing and to explore the health consequences of digital marketing and emerging technologies such as mobile and location-based marketing,' says Dr Anasuri. Such research could help to determine standards for marketing foods and beverages to children and young adults.

'Nutritional interventions must help young adults connect with their peers, as they are the source of their identity, self-concept, friendship, independence, and authority,' continues Dr Anasuri. 'When supported by their friends, teens are more likely to practice fitness and healthy eating.'

Rather than social media having a negative impact on people's health and wellbeing, influencing them to eat unhealthy foods, many educators have begun to consider social media as a potential tool for better engaging participants and communicating beneficial information to encourage healthy lifestyles. In the future, Dr Anasuri's investigations and analyses could be used to develop a set of general guidelines, informing the decisions of policymakers and assisting them in developing more effective strategies to foster healthier lifestyles and eating habits in young generations.

Meet the researcher

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Dr Sadguna Anasuri is an Associate Professor and Program Coordinator at Alabama A&M University. She holds a BSc in Home Science from the College of Home Science in Hyderabad, India, an MSc and an MS in Human Development & Family Studies from the Acharya N. G. Ranga Agricultural University and the University of North Texas, respectively, and a PhD in Child Development from Texas Woman's University. After completing her studies, Dr Anasuri pursued teaching positions at several universities, both in the US and India. Throughout her career, she has carried out research focusing on a variety of topics, including diversity among children and families, resilience across the lifespan, childhood poverty, grandparents raising grandchildren, language and intelligence in children, and the impact of the media on families. Her work has been published in several reputable scientific journals and presented at numerous conferences across the US. Over the years, she has worked closely with children, adolescents, young adults, and older adults in different settings – as an instructor, a researcher, supervisor, mentor, and an academic advisor. Dr Anasuri is also a Certified Family Life Educator and has been a member of numerous committees and associations, including the National Council on Family Relations, American Association for Family and Consumer Sciences, Family Relations, Journal of Educators Online, Society for the Teaching of Psychology, and American Psychological Association, Division II.

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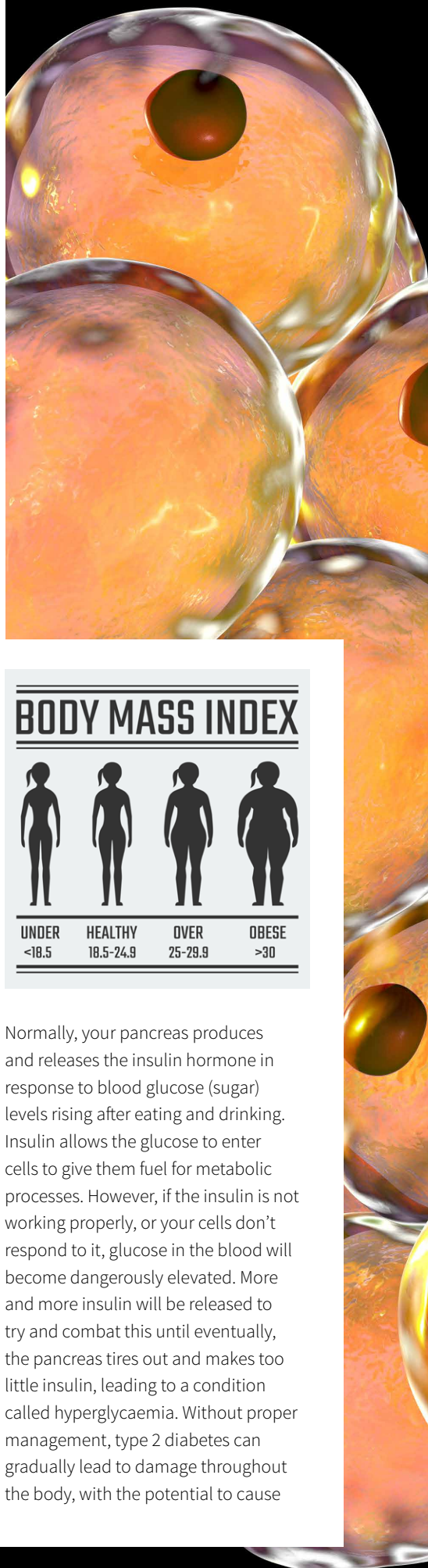
THE ROLE OF TRANSCRIPTION FACTORS IN CHRONIC ADIPOSE TISSUE INFLAMMATION

A transcription factor known as Early B-Cell Factor 1 (EBF1) is key to the formation of fat cells, called adipocytes. Although it is also active in mature adipocytes, the function of EBF1 at this stage has been unclear. **Dr Michael Griffin** at Sam Houston State University in Texas is investigating how EBF1 is involved in the process of adipose tissue inflammation caused by obesity. This type of inflammation is believed to be the underlying cause of a multitude of diseases ranging from diabetes to cancer.



Obesity Worldwide

The World Health Organisation estimates that globally, around 40% of people are overweight and 13% are obese. Defined as having an excess of fat that can have adverse effects on health, an overweight or obese diagnosis is usually given after a person's body mass index (BMI) is shown to be over 25 or over 30, respectively. BMI is calculated by dividing a person's weight by the square of their height in meters (kg/m2). With prevalence in under 19s rising from 4% in 1975 to 18% in 2016, more deaths are now linked with being overweight than underweight worldwide.

Gaining excessive weight occurs when calories, or energy intake, is greater than energy expended. Commonly, this is down to too little physical activity and consuming fat-rich and sugary food and drinks, which are more readily available than they used to be. The modern age has introduced more sedentary jobs and easier access to transportation, both of which decrease a person's calorie expenditure, and this may explain the rise in obesity levels.

Risks of Obesity

Being overweight can give rise to numerous health consequences – cardiovascular diseases, such as strokes, were the number one cause of death globally in 2012. Other risks include musculoskeletal disorders like osteoarthritis, numerous cancers like those of the liver, kidney and gallbladder, and in addition, type 2 diabetes mellitus.

There are two types of diabetes – type 1 diabetes is an autoimmune disorder, which causes your body to destroy the cells in the pancreas that make insulin. Type 2 diabetes is a condition that begins when, for any number of complex reasons, tissue cells like muscle and fat become resistant to the actions of insulin. Eventually, this wears out the pancreas, whereby it doesn't make enough insulin, or it becomes ineffective. This form, generally caused by being overweight, accounts for 90% of all diabetes cases.

Normally, your pancreas produces and releases the insulin hormone in response to blood glucose (sugar) levels rising after eating and drinking. Insulin allows the glucose to enter cells to give them fuel for metabolic processes. However, if the insulin is not working properly, or your cells don't respond to it, glucose in the blood will become dangerously elevated. More and more insulin will be released to try and combat this until eventually, the pancreas tires out and makes too little insulin, leading to a condition called hyperglycaemia. Without proper management, type 2 diabetes can gradually lead to damage throughout the body, with the potential to cause

debilitating or even life-threatening ailments. Damage to the kidneys, eyes, feet, gums and other organs may all occur if blood sugar levels are not properly controlled.

Sufferers of type 2 diabetes may experience fatigue, unusual thirst, slow-healing injuries and blurred vision, but their symptoms tend to be easier to manage than those with type 1. A balanced diet and active lifestyle are great ways to combat type 2 diabetes as well as obesity, but medication and insulin injections can also be given.

EBF1 in Adipocyte Inflammation

Another potential consequence of obesity is fat cells (adipocytes) becoming inflamed. Inflammation is a natural immune response to harmful stimuli such as injury and infection, with the goal of protecting the body. Various chemicals, antibodies and proteins are released and sent to the affected site, in addition to increased blood flow. In healthy responses, inflammation is acute, meaning it is confined to the area of concern and dies down once the threat has passed. However, when this does not happen, perhaps because the trigger has not been resolved, inflammation can become chronic.

Chronic inflammation of adipose tissue may be an underlying cause of many of the health issues that can accompany obesity, such as tissue damage, insulin resistance, diabetes, cancer and others, because they often go hand-in-hand. In fact, it has been shown that people who are obese typically have two to three times more immune factors (small molecules involved in the immune and inflammatory response) in their blood plasma than what is considered normal. Adipocytes and the mechanisms by which inflammation occurs in them is the focus of Dr Michael Griffin's research.

Based at Sam Houston State University in Texas, Dr Griffin and his team have gained novel insights into the biological pathways in adipocytes. He and his team determined that a promising target for investigation was a transcription factor called Early B-Cell Factor 1 (EBF1).

Transcription factors are proteins that control the first step of decoding the genome and making proteins that carry out the function of cells – transcription. Binding to a specific DNA sequence, they determine whether or not individual genes are allowed to be transcribed, which ensures the genes are expressed at the right time, place and amount. EBF1 was already known to be important in the formation of adipocytes, but very little information was available on why it can be found at high levels in mature cells and therefore which pathways it might regulate that lead to inflammation. To rectify this, the team set out to investigate the function of EBF1 in mature adipocytes and its targets for regulation.

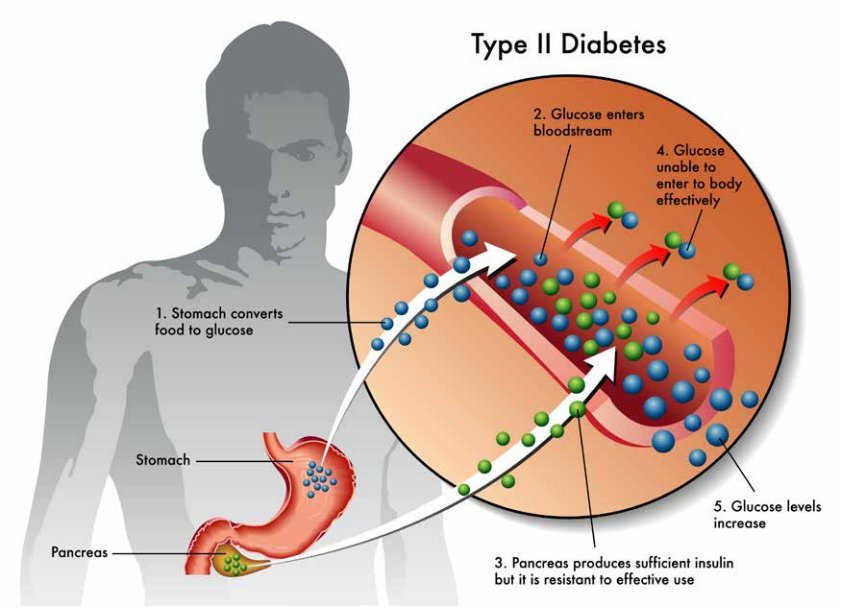
Investigating EBF1 Regulation and Function

The team utilised two techniques in their research – transcriptional profiling and genome location analysis. Transcriptional profiling, also known as expression profiling, measures the expression of genes in a cell. When a gene is expressed, it means that it has gone through transcription to produce a small RNA strand which will go on to code for a protein. Therefore, to find out which genes are being expressed in a specific cell, you can measure how much of their consequent RNA is present in it.

Dr Griffin used this technique in adipocytes that had had the genes coding for the EBF1 knocked out (removed), resulting in cells that were deficient in the transcription factor. This meant that they could decipher which genes it controls and normally allows to be expressed. They also used genome-wide location analysis to establish where the genes that code for EBF1 are found on the genome.

He identified around 35,000 places where EBF1 binds to DNA to aid gene regulation. A few thousand of these sites are specific to adipocytes, meaning they are not found in other types of cells. Most of the sites that EBF1 occupies are called enhancers, which are small sections of DNA that promote (increase the likelihood) of a particular gene being transcribed when a transcription factor is bound to it.

In addition to these findings, the team found that in cells that lacked EBF1, the signalling from multiple metabolic and inflammatory pathways was reduced, suggesting that the transcription factor directly regulates their components. Some of the processes affected included the PI3K/AKT pathway, which regulates the cell cycle and survival, the MAPK pathway, which reads external signals to elicit appropriate responses and, the STAT1 pathway, which is involved in the immune response.



In total, Dr Griffin observed that the expression of 600 genes was altered by knockout of EBF1 in mature adipocytes. Many of these genes encode important intermediates in various signalling pathways, interestingly, including ones involved in inflammatory responses. The team also noticed a reduction in glucose uptake into cells in response to insulin, as well as diminished lipogenesis (fat creation). Overall, they concluded that EBF1 in fat cells plays an important role in regulating inflammation and insulin signalling.

Digging Deeper

The results of Dr Griffin's initial study have helped us to understand the role of EBF1 in adipocytes and how it utilises transcription regulation to alter a number of signalling pathways as well as inflammation.

Now, Dr Griffin wants to focus his attention on digging deeper into this topic and finding out how EBF1 regulates inflammatory signalling in adipocytes. He has uncovered novel protein-protein interactions between the EBF1 protein and another well-known inflammatory transcription factor. This means that inflammatory pathways that we are already aware of are likely to kick off EBF1's role in inflammation of adipocytes. It also suggests that the development of drugs designed to block these interactions could one day represent a viable treatment for Type 2 diabetes associated with obesity.

Therapeutics for Multiple Diseases Associated with Obesity

There is substantial evidence that chronic inflammation of adipose tissue as a result of being overweight is likely to be the root cause of many subsequent diseases (diabetes, heart disease, cancer etc). If the mechanisms behind this inflammation can be fully understood, the development of therapeutics that combat multiple obesity-caused diseases at the same time, is possible.

Designing drugs that utilise this information, especially regarding EBF1, may be an exciting avenue to explore in the future. A healthy diet with a good amount of exercise is always beneficial for treating obesity. But taking into account knowledge of inflammation could aid the prescription of personalised diets and exercise regimes, to better the quality of support and care of those who experience obesity. Dr Griffin hopes that understanding the inflammation pathways regulated by EBF1 will aid in the development of future therapeutics with the potential to treat multiple illnesses at once.

Meet the researcher

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Dr Michael Griffin gained his Bachelor of Science in Nutrition Science from Pennsylvania State University in 1998. He then went on to the University of California at Berkeley, where he achieved a PhD in Biochemical and Molecular Nutrition. Having served in research, mentoring and teaching posts across the USA, Dr Griffin is now Assistant Professor of Biochemistry and Molecular Biology at Sam Houston State University College of Osteopathic Medicine in the Department of Molecular and Cellular Biology. This is also where he carries out his research into type 2 diabetes, which focusses on the role of inflammation of adipocytes (fat cells) in insulin resistance.

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Sam Houston State University

UNDERSTANDING THE CAUSES OF PANCREATIC DISEASE TO IMPROVE PATIENT OUTCOMES

The incidence of pancreatic disease, including pancreatitis and pancreatic cancer, is on the rise, but currently, preventative measures and effective treatments are scarce. **Dr Stephen Pandol** at the Cedars-Sinai Medical Center in Los Angeles is working to change this. Dr Pandol carries out broad and far-reaching research, ranging from how lifestyle factors impact pancreatic disease to the molecular and cellular mechanisms behind pancreatic cancer resulting from obesity. His dedicated work has led to significant progress in the field and is driving forward the potential for better patient outcomes.

The Function of the Pancreas

The pancreas is a roughly hand-sized organ found just behind the stomach. It has an integral role in both exocrine function during digestion and endocrine function for blood sugar regulation.

Its exocrine function involves creating around 200 ml of digestive juice daily, which is packed with enzymes like protease, lipase and amylase to break down proteins, fats and sugars. The juices travel through the ducts in the pancreas to the duodenum in the small intestine where they get to work digesting meal nutrients for absorption.

Pancreatic hormones for endocrine function are also synthesised in the organ but are released into the bloodstream to send signals to other organs. One hormone, insulin, is synthesised by pancreatic cells called beta cells, which make up around 75% of the hormone cells in the pancreas. When an increased level of glucose is detected in the blood after eating, insulin sends messages for it to be stored in the liver where it can be used later. Glucagon is another hormone that signals for this stored sugar to be released by the liver when blood glucose levels are low. It is made in alpha cells, which comprise around 20% of the hormone cells.

Diseases of the Pancreas

A well-known pancreatic disease is diabetes. Type 1 diabetes is an autoimmune disease that results in the destruction of insulin-producing beta cells and a subsequent inability to regulate blood sugar. On the other hand, type 2 diabetes is often a consequence of obesity. Constant high blood sugar levels cause cells (such as in the liver) to become resistant to insulin and so increasingly higher amounts of the hormone are produced to try and fulfil the need. However, this overproduction is unsustainable and eventually, beta cells burn out and can't make enough insulin. Both Type 1 and Type 2 diabetes have the outcome of elevated blood sugar which can be remedied through medications, and for type 2 diabetes, diet management.

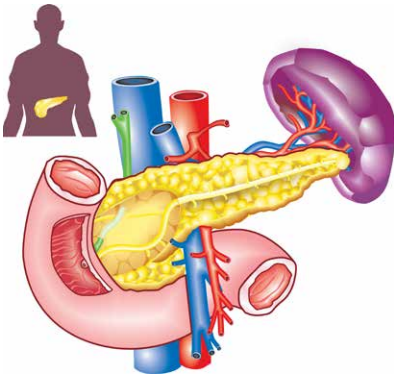

Illustration of the pancreas

Pancreatitis is a painful inflammation of the pancreas caused by digestive enzymes attacking the organ itself. It can be caused by gall stones or excessive alcohol consumption and may lead to malnutrition and weight loss.

Another health issue that can arise within the pancreas is pancreatic cancer. Around 95% of pancreatic cancers start in exocrine cells and are called pancreatic adenocarcinomas. It causes a reduction in the number of digestive enzymes produced, and as a result, loss

of appetite and weight loss are common indicators of the disease. Risk factors for pancreatic cancer include obesity, diabetes smoking and chronic forms of pancreatitis.

Pancreatic Adenocarcinoma is a Significant Health Dilemma

In a world where overall deaths due to cancers are decreasing, incidence and deaths from pancreatic cancer are still on the rise. In the last 30 years, the five-year survival rate has shockingly only increased from 3% to 7%. Contributing to this poor figure is the fact that only 20–30% of pancreatic cancer patients are eligible for resection (removal of the tumour) because the disease is often only detected after it has widely spread. This surgery is currently the best chance of cure and even these patients can relapse. Inadequate understanding of markers that could lead to earlier diagnosis and ineffective treatment regimens both contribute to these harsh outcome numbers.

However, determined scientists like Dr Stephen Pandol are leading the way in progressing understanding of pancreatic disease. At the Cedars-Sinai Medical Center in Los Angeles, Dr Pandol explores the underlying mechanisms of pancreatic disease and cancer, with a particular focus on lifestyle factors. The overarching goal of this work is to develop more effective therapeutics.

Obesity as a Pancreatic Cancer Risk

Diet-induced obesity and chronic forms of pancreatitis are frequently connected with the development of cancer, including pancreatic adenocarcinoma. However, the processes that lead from them to cancer are unclear. Working with colleagues at Cedars-Sinai Medical Center and the University of California, Los Angeles (UCLA), Dr Pandol examined these associations using mice.

They used wildtype mice, which are mice with standard genetic material, and KC mice, which are mice with a pancreas-specific oncogenic mutation in their Kras gene. Both mice were fed a high-fat and high-calorie diet so that they developed diet-induced obesity. The wildtype mice exhibited the expected weight gain and insulin resistance. Dr Pandol and his team focused on the role of cells in the microenvironment of the pancreas known as pancreatic stellate cells (PaSCs). These cells mediate the development of fibrosis and inflammation that occurs in pancreatic cancer and interact with early pancreatic cancer cells to facilitate the progression and metastasis of the cancer. All of these factors are linked to the acceleration of the development of pancreatic adenocarcinoma tumours, which implies that pancreatic stellate cells have an important role in promoting obesity-induced pancreatic tumours.

Dr Pandol and the team found that numerous signals are a crucial element in activating the pro-cancer effects of PaSCs. These included extracellular signals such as the cytokines IL-4/

IL-13 and the hormones insulin and insulin-like growth factor 1 (IGF1). Intracellular signals are also needed – transcription factors like STAT3 and enzymes like mTOR/Akt. By regulating the immune system to favour the tumour, preventing apoptosis (cell death) and promoting tumour cell growth, these signals allow PaSCs to continue supporting tumour progression.

The team also analysed large databases of people who had experienced pancreatic adenocarcinoma, and specifically, patients who received simvastatin as a therapy. Simvastatin is a member of a group of drugs that lower cholesterol, called statins. It can be prescribed to prevent cardiovascular issues or given as a treatment for type 1 and type 2 diabetes. Patients in this retrospective analysis showed better disease-free survival rates after tumour resection if they took simvastatin.

Summarising these studies, Dr Pandol hypothesises that obesity produces unique signals in the microenvironment of developing pancreatic adenocarcinomas, inducing observable alterations in PaSCs. The result is the production of signals that promote rapid growth and resistance in cancer cells, and a shift in the immune response to a pro-tumour state. In other words, obesity creates a much more favourable environment for cancer cells to survive and spread.

Building on the Findings

This work is only the beginning of what Dr Pandol plans to achieve and he has specific aims for his future work. He plans on studying the effects of eliminating activated PaSCs at various stages of pancreatic adenocarcinoma development in KC mice who will be fed normal and high obesity-inducing diets. He will also continue work to uncover how different cell types 'talk' to each other within a tumour microenvironment. This will provide insight into the role of PaSCs in tumour development.

Additionally, determining which pathways obesity impacts to promote cancer formation through PaSCs is important. Further studying how the previously mentioned signals (such as IL-4) and their pathways respond to obesity will shine light on how processes leading to the activation of PaSCs are involved in cancer.

Simvastatin and another drug known as metformin are also interesting leads to explore. Metformin reduces the amount of sugar being released from the liver whilst improving insulin's function to remove sugar from the blood. This makes it a common and effective way of reducing blood glucose levels for patients with type 2 diabetes. Dr Pandol and his team will study how these drugs affect the activation of PaSCs and tumour progression in KC mice. The findings will be used to improve preventative strategies for at-risk patients, and are likely to play an essential role in improving pancreatic cancer survival rates.

Contributing to the Field

All of this is just a fraction of the work that Dr Pandol has headed and contributed to over his extensive career in pancreatic disease research. He has studied how stressors on pancreatic organelles lead to pancreatitis, investigated how pancreatic disease diagnosis can be improved through developing magnetic resonance imaging techniques, and much more. Upcoming studies will look into how lifestyle factors such as alcohol consumption and smoking, lead to pancreatitis so that clinical recommendations can be updated. His work is proving to be vital for broadening and deepening our understanding of pancreatic disease so that patient outcomes can be vastly improved.

Meet the researcher

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Dr Stephen Pandol completed his Bachelor of Science in Biochemistry at the University of California, Davis, and went on to achieve his Doctor of Medicine degree at St. Louis University in Missouri. Dr Pandol is extremely active in his clinical and research work on pancreatic biology and disease, and as part of his remit, directs the Basic and Translational Pancreatic Research at Cedars-Sinai Medical Center He also co-directs the UCLA Center for Excellence in Pancreatic Diseases, the Southern California Research Center for Alcoholic Liver and Pancreatic Diseases and the NIH-sponsored Consortium on Chronic Pancreatitis, Diabetes and Pancreatic Cancer. In addition, he is a Staff Physician, Professor of Medicine and past President of the American Pancreatic Association, from whom he received a Lifetime Achievement Award in 2015.

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DETERMINING THE LINK BETWEEN DIET AND CANCER

Cancer is a leading cause of death worldwide and understanding the development of the disease is essential for prevention and treatment. **Dr T. Colin Campbell** from Cornell University's Division of Nutritional Sciences proposes the intriguing theory that cancer is not primarily a genetic disease but a nutrition-responsive disease. By conducting numerous animal and human studies, he is providing convincing evidence on the importance of diet, particularly the consumption of animal-based protein, in the development of cancer.

Challenging the Current Understanding of Cancer

Cancer is a leading cause of morbidity and mortality worldwide and is responsible for approximately 9.6 million deaths globally per year according to the World Health Organization. A better understanding of the disease is necessary to ensure the provision of targeted and effective treatment as well as to prevent its onset.

Cancer is typically considered a genetic disease, starting with a gene mutation in the DNA of a cell which can be inherited or acquired. Acquired mutations occur as a result of environmental cancer-causing agents (carcinogens) which are activated by enzymes within the body to give products that subsequently bind to and alter the DNA, causing genetic mutation. As cells with mutated DNA continue to multiply, more mutations develop and the cells begin to display characteristics typically observed in cancer, such as limitless replication, evasion of cell death, and drug resistance.

It is believed that these mechanisms by which cancer occurs are irreversible. Current cancer treatments, such as surgery and chemotherapy, are based on this theory of irreversibility and aim to remove or kill cancer cells, rather than reverse their pathogenesis.

Dr T. Colin Campbell in the Division of Nutritional Sciences at Cornell University refutes this theory of cancer development, deeming it as 'reductionist'. His research is demonstrating that cancer is not merely a genetic disease, but rather, that nutrition controls the expression of these genes in the development of cancer and possibly suspend and even reverse further development. Over the past six decades, Dr Campbell has examined the existing evidence for a relationship between nutrition and cancer and elucidated the optimal diet for the prevention of cancer.

Examining the Existing Evidence

In a 2017 publication, Dr Campbell delved into the history of research surrounding the association between diet and cancer, which appeared

as early as the 1800s. He discusses more than a century of work in which numerous researchers cite a diet rich in protein (particularly meat), fatty foods, and a lack of fruit and vegetables as a primary cause of cancer. Some reports concluded that diet was responsible for a large proportion of avoidable cancers.

Despite this long-standing research, progress in understanding the role of nutrition in cancer has been slow and these conclusions have largely been forgotten or overlooked. Dr Campbell attributes this to a reluctance to

challenge the widely accepted genetic theory of cancer, resistance from the food industry, and because 'nutrition is not respected as a legitimate science, especially in medical practice communities'.

Two relatively recent periods of nutrition research have revolved around the potential of specific vitamins to prevent nutritional deficiency diseases and chronic diseases such as cancer. However, in trials investigating this, nutrients are examined in isolation, usually in the form of supplements. In doing so, the complex interactions of nutrients with other compounds in whole foods are ignored and, as Dr Campbell explains, 'isolated nutrients, when consumed as supplements for example, often do not have the same functions as they do when present in whole food'. Dr Campbell critiques this reductionist view of nutrition and believes it has led to a diversion of attention away from the true potential of whole foods to combat disease.

The association between diet and other diseases such as diabetes and cardiovascular disease has been well documented. Furthermore, it has been observed that when people transition from simple diets of rural cultures to Western diets that are high in fat, rates of heart disease, diabetes and cancer increase. This association has been attributed to the consumption of dietary cholesterol and saturated fat in high-fat diets. However, this theory overlooks the fact that cholesterol and saturated fats are primarily found in animal-based foods. Dr Campbell suggests that the culprit of the disease-promoting diet is not cholesterol and saturated fat, but instead, the consumption of animal-based foods obviously characterised by protein.

This theory arose from an observational finding during a programme in the Philippines coordinated by Dr Campbell that was designed to resolve childhood malnutrition by ensuring sufficient protein consumption. However, Dr Campbell found that the children at higher risk of dying of liver cancer tended to come from the wealthiest families – those that consumed more animal protein.

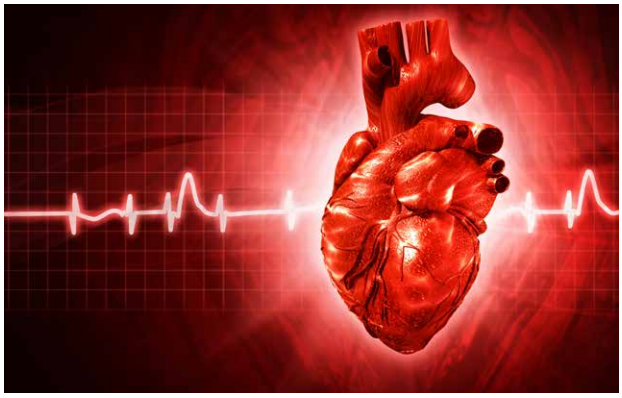
Investigating the Role of Diet and Nutrition

To investigate the effect of animal protein consumption on cancer development, Dr Campbell and his team conducted a series of laboratory experiments on animal models in which cancer had been induced by aflatoxin, a potent carcinogen. Cancer grew when animals were fed a diet in which protein comprised 20% of total calories, but growth was repressed when animals were fed a diet in which protein comprised just 5% of total calories. Switching between 20% and 5% protein diets successively turned on and turned off tumour development during the first 12 weeks of pre-cancerous growth in animal models.

Furthermore, in a lifetime study on rats and mice conducted over 2 years, by the end of the study, all animals on a 20% protein diet had died as a result of liver cancer whereas all animals fed a 5% protein diet were alive and without liver cancer. The protein used in these studies was casein, the main protein in cow's milk. The same tests were conducted using vegetable protein rather than animal protein and the researchers found that even at 20%, the vegetable protein diet did not induce any pre-cancerous cells.

On the basis of these studies, Dr Campbell and his colleagues concluded

'My main finding of most relevance, based on epidemiological and clinical findings on humans, mechanism studies on disease formation in experimental animals, and research findings of others during the past two centuries, is that the human diet ideally should rely on the consumption of whole, plant-based foods.'



that cancer development was responsive to nutritional exposure to protein, in both directions, at the early and late stages of cancer development. Describing the findings of his work, Dr Campbell explains that 'experimental cancer development could be turned on and off by increasing and decreasing, respectively, animal protein consumption'.

To explain the mechanisms behind these findings, Dr Campbell and his team conducted further experiments in rats and mice. They found that dietary protein intake was positively correlated with the amount and the activity of the principal enzyme that activates carcinogens. Dietary protein also increased the chemical bonding of carcinogen with DNA, leading to genetic mutations. In conclusion, the researchers found that high dietary protein of animal origin up-regulated mechanisms that increase cancer development and down-regulated the cell's normal ability to reverse development.

These experiments were particularly noteworthy because it demonstrates that cancer causation involves multiple mechanisms working in synchrony to cause the cancer response. In a human setting, when consumption of animal-based protein increases, plant-based protein decreases, also relying on the same multiplicity and synchronicity of mechanisms, thus accentuating the apparent animal protein effect on cancer development.

Dr Campbell and his team have also conducted several human population studies to investigate the role of diet in cancer, the most notable of which is the China Study. This study is regarded as one of the most comprehensive population studies ever conducted and comprised a 65-county, 130 village cohort in rural China for which data were collected in 1983 and again in 1989.

It was discovered that the incidence of primary liver cancer was not associated with aflatoxin exposure. Instead, the most highly correlated lifestyle factor with liver cancer was the level of blood cholesterol, which itself is associated with greater consumption of animal-protein based foods. The researchers concluded that counties with higher consumption of animal-based foods (still only about 10% of Western consumption) were more likely to have higher death rates from 'Western' diseases, while the opposite was true for counties that consumed more plant-based foods – quite a condemnation of consuming animal-based food. Plants provide all the protein we need, oftentimes even more.

Seeking a More Effective Strategy for Cancer Prevention and Treatment

Dr Campbell hypothesises that cancer is primarily a nutrition-responsive disease, an intriguing theory that has been met with controversy. He advocates a whole-food, plant-based (WFPB) diet to protect against an array of diseases, including cancer. As he states, 'my main finding of most relevance, based on epidemiological and clinical findings on humans, mechanism studies on disease formation in experimental animals, and research findings of others during the past two centuries, is that the human diet ideally should rely on the consumption of whole, plant-based foods'. Very few studies have investigated the impact of a WFPB diet compared to animal protein-based diets on the prevention of disease, though evidence to date also supports the reversal of heart disease, diabetes and chronic kidney disease. Dr Campbell states that more intervention trials in human patients are required to test his broad-based hypothesis.

Given that current cancer treatments are costly to develop and often have negative side effects, a whole food-nutritional approach to cancer prevention and treatment could be more effective. Dr Campbell encourages the rejection of reductionist theories of cancer and nutrition and argues for a better understanding of the complexities of cancer development and nutrient interactions within whole foods to map a more effective strategy to cancer prevention and treatment. Dr Campbell's recently published book, 'The Future of Nutrition: An Insider's Look at the Science, Why We Keep Getting It Wrong, and How to Start Getting It Right', provides a stimulating account of his perspectives on nutrition and health.

Meet the researcher

Dr T. Colin Campbell
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Dr T. Colin Campbell was the first in his family to attend college, obtaining his PhD in nutrition, biochemistry, and bacteriology from Cornell University. Following ten years as a faculty member at Virginia Tech University, he was recruited back to Cornell University in 1975 as a professor with tenure where he currently holds an endowed chair as the Jacob Gould Schurman Professor Emeritus of Nutritional Biochemistry in the Division of Nutritional Sciences. His research focuses on the relationship between diet and disease and comprises both laboratory experiments and large-scale human studies, including The China Study; the most comprehensive epidemiological study on nutrition ever conducted. As a result of his extensive research into nutrition and health over six decades, Dr Campbell has received multiple lifetime achievement awards as well as several humanitarian, courage, and related awards. Dr Campbell strives to communicate evidence-based information on nutrition and health.

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FUNDING

National Cancer Institute of the NIH
US State Department
American Cancer Society
American Institute for Cancer Research
Imperial Cancer Research Fund (UK)

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INFECTION AND THE IMMUNE SYSTEM

FIGHTING INFECTION AND DEFENDING AGAINST DISEASE

Following the sudden emergence of COVID-19 which led to a worldwide pandemic, we are perhaps more aware than ever of the risks of potentially fatal infection. The third section of this issue of Scientia focuses on the researchers progressing knowledge about how our bodies fight disease and infection, and the important consequences of this for medical science and healthcare delivery.

Bacterial infections are common and caused by the transmission of bacteria (for example, from other people or eating contaminated food). Many bacterial infections need to be treated with prescription antibiotics and if left untreated, can be fatal. Antibiotics underpin most of modern medicine but the rapid rise of resistance to these drugs presents an imminent global health disaster if not adequately managed. We open this section with an exclusive interview with Professor Colin Garner, founder and Chief Executive at Antibiotic Research UK. We read how the charity supports research into new antibiotic treatments, educates the public about the dangers of drug-resistant infections and provides the UK's first patient support service for patients with resistant infections.

We then turn to Dr Bert Lampson at East Tennessee State University, who has dedicated his lifetime research career to understanding the dangers and applications of bacteria. We read how he has discovered new mechanisms of antibiotic resistance, novel antibiotics and bacterial proteins, all with important applications for science and healthcare. Interestingly, his work also shows that although bacteria can cause harmful, resistant antibiotic-resistant infections, they also harbour proteins with useful applications that can be exploited and bring important benefits.

Bacterial infections cause most cases of sepsis, a severe and life-threatening response to infection in the bloodstream. The early detection of sepsis is a complex but critical diagnostic task. Dr Richard B. Brandon, Dr Thomas D. Yager and their colleagues at Immunexpress have developed a molecular diagnostic platform capable of detecting genetic biomarkers diagnostic for sepsis in around only one hour. We read how this innovation can lead to better patient management, more efficient use of resources, and better use of antibiotics.

The rise of pandemic-causing viruses such as COVID-19 is an unfortunate by-product of our hugely connected world. Dr Babita Agrawal from the University of Alberta at the helm is playing a key role in the move towards a new era of preventatives and therapeutics to help us fight disease. We read how Dr Agrawal and her team are investigating novel vaccines and immunotherapeutics for influenza, Hepatitis C, tuberculosis, and even cancer.

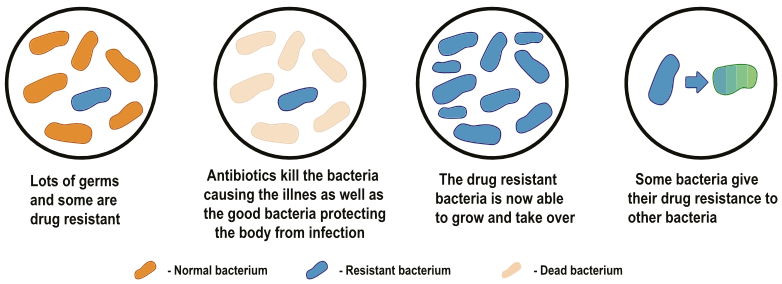
Many infections can trigger oxidative stress, an imbalance between free radicals and antioxidants in the body. Professor Marino Resendiz at the University of Colorado Denver studies how modifications generated by oxidative stress alter the function and structure of RNA, an important component of all cellular organisms. We read how he has demonstrated some of the changes that oxidative damage can result in, and how his work may lead to the identification of novel structures within cells that present potential therapeutic applications.

We conclude this section by meeting Professor Hani El-Gabalawy at the University of Manitoba. Rheumatoid arthritis is an autoimmune disease in which the immune system (which usually fights infection) attacks the body's own cells, causing progressive damage to tissues and organs. The indigenous North American peoples exhibit disproportionately high rates of the disease, and we read of Professor El-Gabalawy's critical work improving the identification of those at risk, aiming to facilitate the development of preventative strategies.

ANTIBIOTIC RESEARCH UK

While antibiotics have transformed modern medicine, helped to extend life expectancy in the UK by as much as 20 years and saved millions of lives around the world, the rapid rise of resistance to these drugs presents an imminent global health disaster if not adequately managed in the very near future. In this exclusive interview, we speak with **Professor Colin Garner**, founder and Chief Executive of Antibiotic Research UK, the world's first charity focussing on bacterial antibiotic resistance, to hear about their vital efforts targeted at overcoming the challenge of antibiotic resistance.

HOW ANTIBIOTIC RESISTANCE HAPPENS



To begin, can you tell us more about why antibiotic resistance is such a serious and urgent problem?

Bacterial antibiotic resistance is a natural phenomenon; in fact, resistant bacteria have been identified in Egyptian mummies long before the advent of antibiotics. Resistance is driven by the misuse and overuse of antibiotics, causing bacteria to evolve metabolic pathways to avoid the killing effects of antibiotics.

Antibiotics underpin most of modern medicine. Without effective antibiotics, life-saving treatments for cancer, heart disease, joint replacement, organ transplantation and even childbirth would become much riskier – if not impossible. Without effective antibiotics, we would go back to a pre-antibiotic age when even a simple scratch could kill. Antibiotic resistance, where bacteria become resistant to antibiotic treatments, is on the rise around the globe. Today approximately 700,000 people a year die around the world from antibiotic-resistant infections. It has been proposed that if we don't deal with antibiotic resistance now, by 2050, 10 million people will die globally each year from a drug-resistant infection.

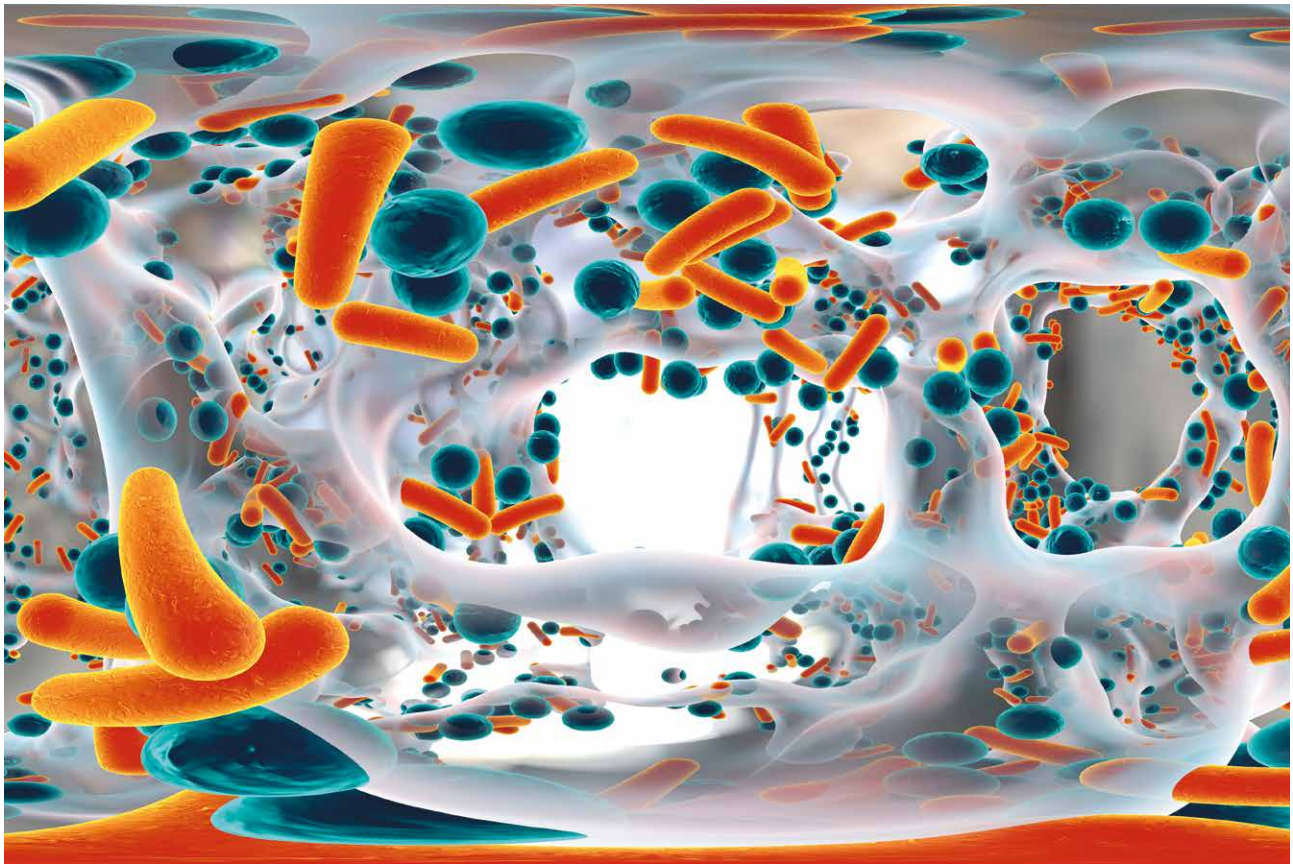
What are the main aims of Antibiotic Research UK?

Antibiotic Research UK (ANTRUK) was formed in 2014 by a concerned group of expert Antimicrobial Resistance (AMR) scientists and clinicians who felt that not enough attention was being paid to this problem in comparison with other diseases such as cancer. The charity takes a holistic view of the problem. Our primary goals are to support research into new antibiotic treatments, educate the public about the dangers of drug-resistant infections and provide the UK's first patient support service for patients with a resistant infection. All the charity's activities are supported by public donation; we receive no government funding.

How does Antibiotic Research UK support research? What types of research do you fund?

Since the charity's inception we have taken the view that, as we are working with limited funds, we should focus on specific areas. For this reason, we decided to concentrate our research efforts on gram-negative bacterial infections, the most common cause of urinary tract infections, pneumonia and bloodstream infections. Multidrug-resistant gram-negative infections are on the rise around the world, including here in the UK. Our funding consists of both commissioned research on, for example, antibiotic resistance breakers, ß-lactam antibiotics/ß-lactamase inhibitors and non-antibiotic alternatives to treat infection.

Through response mode funding we have funded a Small Research Grant programme in which UK academics applied on a competitive basis for funding. To date, we have funded ten Small Research Grants but because of COVID, we had to postpone our 2020 call for proposals. Funding permitting, we hope to have a further call in 2021.



How is the general public involved in your work?

As a charity, the public are our primary stakeholders and funders. Our charity is what is known as a Charitable Incorporated Organisation with members. This means, rather like a company with shareholders, our charity is controlled by our members who can join for a small membership fee.

We are currently in the process of reaching out to the public to gain a better understanding of their knowledge of antibiotic resistance to ensure we provide the most beneficial information in an easy to access format. If anyone feels that they want to see their children and grandchildren enjoy the same benefits of antibiotics that we have enjoyed, they should join 'the resistance against resistance'.

We are, of course, in the midst of the COVID-19 pandemic. What specific challenges and opportunities has this brought to the work of Antibiotic Research UK?

COVID-19 is the most serious health crisis the world has faced in the past 100 years. The word pandemic was unknown to most people until COVID struck. As an infectious disease charity, we no longer have to explain the term but there are still gaps in the public's knowledge.

Some people don't realise the additional risk that COVID-19 patients are exposed to in hospital where, due to their lowered immunity, they are in danger of contracting secondary bacterial infections. The combination of COVID-19 with bacterial pneumonia can be deadly and, to counteract this, many hospital patients are routinely given antibiotics to reduce the likelihood of secondary infection. This is not good clinical practice and should be avoided unless positive identification of a bacterial infection has been made. However, there are no rapid diagnostics for the identification of bacterial infections, making it difficult for ICU clinicians to make quick, informed decisions.

The increased awareness of infectious disease is a rare silver lining in these difficult times and we hope it will result in a re-evaluation by all stakeholders of the importance of fundamental science research. However, the volume of patients and the increased risk to life can make hospitals less conservative with the use of antibiotics. Overuse and improper use of antibiotics is a major driver of antibiotic resistance and may be fuelling the next crisis post-COVID.

In the fight against antibiotic resistance, as with the fight against COVID-19, we must remember our scientists. It is scientists who discovered and developed the COVID vaccines, but all too often these scientists work under very difficult conditions with limited funding. I hope the COVID pandemic will result in much more infectious disease funding including for antibiotic resistance research.

The COVID pandemic has hit our funding hard as face-to-face fundraising events have had to be cancelled making it more difficult for us to carry out our mission. That is why I hope anyone reading this article will help us in our essential work.

What would you say are Antibiotic Resistance UK's biggest achievements to date?

Our main achievement is that we exist! Until our charity was created there was no charity focussing on bacterial antibiotic resistance.

I am extremely proud of our achievements – we have raised nearly £2 million since we were formed in mid-2014 and these funds have been used to support our three goals of research, education and patient support.

I believe we punch above our weight. We have held meetings with Government ministers including the then Prime Minister Theresa May to highlight the critical issue of AMR.

We have also worked with major pharmaceutical companies such as MSD, Shionogi and Pfizer. One research programme we have supported might provide a new treatment for multidrug-resistant Gram-negative infections and so achieving one of our goals of getting a new treatment into the clinic by the early 2020s.

Looking now to the future, what are the main challenges and goals for Antibiotic Research UK over the next 5–10 years?

The main challenge as I see it, is to increase the profile of the resistance problem alongside raising the profile of our charity.

We want to become known as the primary charity in this space providing evidence-based support and information. We have produced a five-point action plan.

In particular, I am keen to see the creation of a UK AMR Research Fund of up to £100 million; the contributors to the fund would be pharma, government and medical research charities. The primary aim of the fund would be to support basic precompetitive science across UK universities and institutes. I would view the creation of such a fund as a major achievement in helping to develop the treatments of tomorrow. I also hope to see our patient support service expanded to serve the over 60,000 people who get an antibiotic-resistant infection every year in the UK.

W: https://www.antibioticresearch.org.uk/
@1Antruk

BACTERIA: DEADLY BUT USEFUL

Bacteria are found everywhere – in humans, animals and the environment – and are best known for being able to cause painful and even fatal infections. It may come as surprise, therefore, to learn that bacteria can also have useful applications. The lifetime work of **Dr Bert Lampson** at East Tennessee State University in the USA has focussed on the dangers and applications of bacteria. Over his career, Dr Lampson has discovered new mechanisms of antibiotic resistance, novel antibiotics, and bacterial proteins, with important applications for science and healthcare.

Revealing Antibiotic Resistance Mechanisms

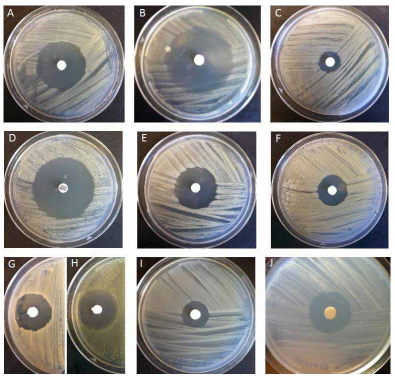
Dr Bert Lampson began his research career as a graduate student in the early 1980s while studying at the University of Missouri-Columbia School of Medicine. At this time, scientists were developing new research technologies, including DNA sequencing. This process involves determining the sequence of nucleotides, the building blocks of DNA. The sequences can tell us what genes bacteria carry. Genes are inherited by bacteria and have many functions, such as encoding proteins that are used within the bacterial cell.

Dr Lampson used DNA sequencing to discover antibiotic resistance genes that are carried by plasmids. Antibiotic resistance genes are genes that encode mechanisms that lead to bacteria becoming resistant to antibiotics. Furthermore, plasmids are small circles of DNA that are physically separate from chromosomal DNA. The chromosomal DNA contains a large number of genes required to maintain cellular functions. In contrast, plasmids can carry a small number of additional genes, also known as accessory genes, that can help bacteria to adapt and survive in their environments. Furthermore, plasmids can be transferred amongst bacteria populations and spread genes. This has led to the spread of antimicrobial resistance genes that prevent antibiotic drugs from successfully treating an infection.

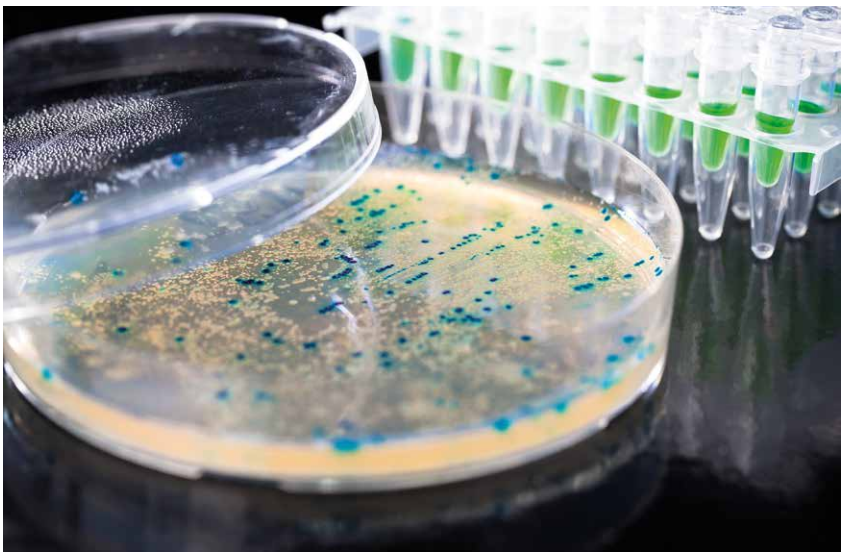
The early work of Dr Lampson first involved sequencing a previously discovered plasmid in Staphylococcus epidermis. This bacterium is found around naturally on the skin. However, when the skin is broken the bacteria can enter the body and cause harmful infections. This particularly occurs when medical equipment, such as needles, are inserted into the body. These infections are most often treated with antibiotics.

Dr Lampson, under the guidance of Dr Joseph Parisi, showed that plasmids can induce resistance to three antibiotics (macrolides, lincosamide and streptogramin). This was demonstrated using transduction (gene transfer between bacteria using a bacterial specific virus called a phage), whereby the plasmids carrying the resistance genes were input into the bacteria not carrying the plasmid. Dr Lampson and his team then treated two sets of bacteria, those with and those without the plasmid, with antibiotics to see if carrying the plasmid led to bacteria becoming resistant.

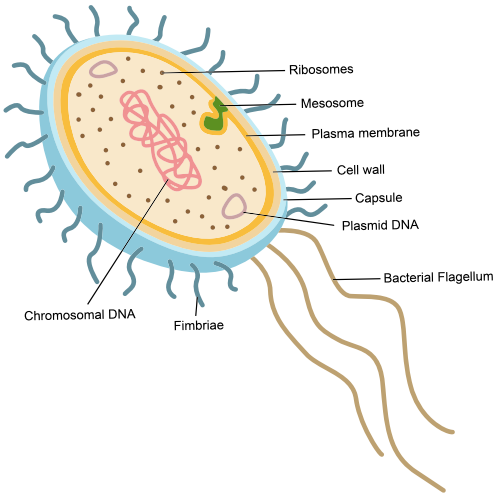
'Wheel' plate containing different Rhococcus bacteria. Credit Bert Lampson.



Spectrum of activity of the novel antibiotic from the Rhodococcus strain. Credit R. Borisova.



STRUCTURE OF A BACTERIAL CELL



Using DNA sequencing Dr Lampson and his fellow researchers pinpointed resistance to be due to a new gene, *ermM*. This gene has a similar sequence to a previously described resistance gene. However, the team observed changes upstream of the gene sequence, leading to changes in how the gene is expressed. *ermM* is constantly 'switched on', meaning it is continually expressed. The gene encodes an enzyme, a protein that is able to help the cell to carry out reactions. Critically, the protein alters the binding site, that is, the target site for antibiotics and prevents them from working.

Remarkably, Dr Lampson also discovered a novel resistance mechanism that is carried by plasmids in the same species of bacteria. The newly discovered mechanism led to resistance to erythromycin. This is an antibiotic that is used to treat a number of infections. It operates by preventing bacterial cells from producing proteins that are essential for life, as well as replication.

Dr Lampson and his colleagues identified a new resistance gene that they designated erpA that was carried on a plasmid pNE24. Experiments that can detect specific DNA sequences, known as southern blot hybridisation, showed that the plasmid carries a new resistance mechanism. This is because the plasmid contains different DNA sequences to other plasmids that also carry erythromycin resistance genes. The new gene makes the bacterial cell less permeable, preventing the uptake of the antibiotic. This was shown by labelling the antibiotic with a radioactive carbon (Carbon-14) which helps scientists to detect the amount of antibiotic that the bacterial cells uptake. This research holds important implications for understanding how bacteria become resistant to antibiotics and how this can spread amongst bacterial populations, particularly in hospitals.

Searching for Novel Antibiotics

When Dr Lampson started his role as a Research Scientist at East Tennessee State University (ETSU), he wanted to work on antibiotics again. However, this time, he took inspiration from work he had completed with the energy company, Energy Biosystems. This company was using an interesting soil bacterium, *Rhodococcus*, to try and produce cleaner fuels but Dr Lampson saw an additional opportunity for this research. Although ETSU is not a primarily research university, Dr Lampson was also keen for students to participate in this exciting new research project.

This work focused on exploiting *Rhodococcus* for antibiotics, which at first, may seem paradoxical. Why would bacteria produce antibiotics that are used to kill them? The reason is that species of soil bacteria have to compete with one another to survive. Therefore, bacteria produce antibiotics to outcompete other species.

Dr Lampson and his students grew *Rhodococcus* on plates that contain agar, on which bacteria can survive. They extracted molecules from these agar plates and then they searched for molecules and proteins from *Rhodococcus* using different forms of chromatography. This process helps to separate the different molecules from one another. After separating the extracts, the team coated small disks with the extracts and tested whether the bacteria could survive in its presence.

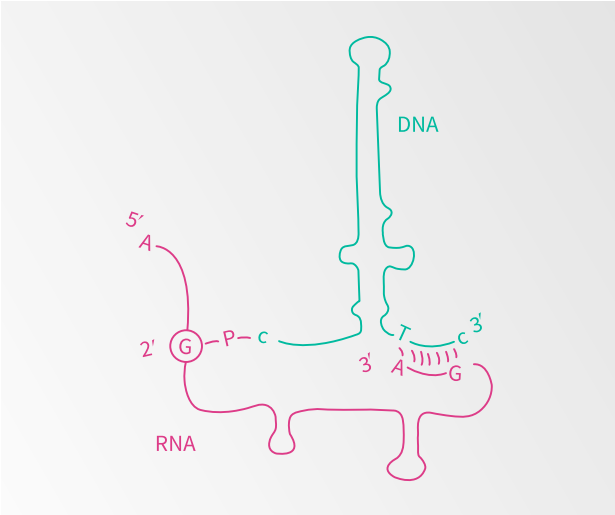
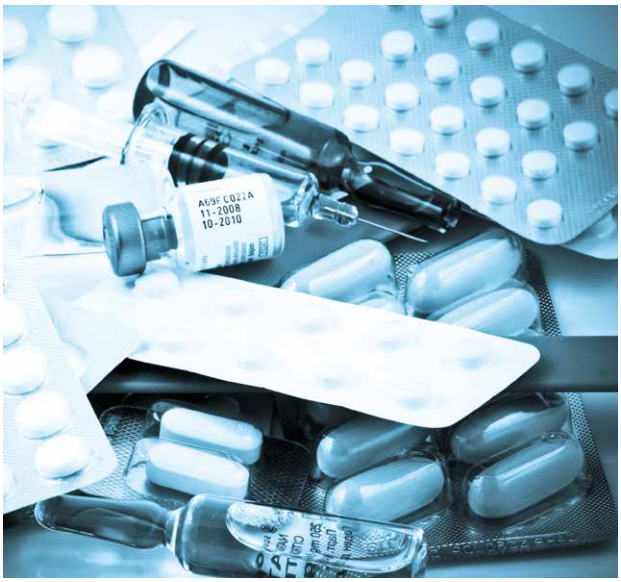


Illustration of msDNA.



This work led to the discovery of a new compound that could inhibit the growth of bacteria with potentially significant applications in medical and veterinary practice. As such, this key study resulted in several more research projects, and towards the end of 2018, a publication for Dr Lampson and his students in the high impact journal PLoS ONE.

Discovering the First Bacterial Reverse Transcriptase

Dr Lampson has additional research interests outside the field of antibiotic resistance. Notably, whilst working as a postdoctoral researcher at the Robert Wood Johnson Medical School, he discovered a protein with very important applications.

Using soil bacteria, this time Myxococcus, Dr Lampson discovered the first example of a bacterial reverse transcriptase. This is a protein, also known as an enzyme, that is responsible for forming DNA from RNA. Generally, people associate DNA with producing RNA and RNA with making proteins. However, this is a simplified view – in reality, the process is much more complex.

Reverse transcription is the formation of DNA from RNA and this takes place in most organisms, including humans and bacteria. Interestingly, it is believed that reverse transcription was a key process in the origins of life and it is hypothesised that RNA was the source of genetic information and cellular functions. This collectively is known as the 'RNA world'.

Dr Lampson, under the mentorship of Professor Masayori Inouye, discovered bacterial reverse transcriptase after the discovery of another strange bacterial element, msDNA. This molecule contains both RNA and DNA bonded together. Dr Lampson and his colleagues showed that this molecule was produced by reverse transcriptase, making this the first reverse transcriptase to be identified in bacteria. Importantly, reverse transcriptase can be used to make DNA from RNA, which in turn means the DNA can be identified using a process known as polymerase chain reaction (PCR) or DNA sequencing. This allows scientists to figure out what DNA is being expressed.

These findings further led to the discovery of a thermostable reverse transcriptase that can operate at high temperatures. High temperatures otherwise alter the structure of a protein and prevent it from functioning. Although reverse transcriptase had been identified from viruses previously, heat stable bacterial reverse transcriptases are more advantageous because they increase the accuracy of DNA synthesis and are potentially more stable under laboratory experimental conditions.

Dr Lampson and his team searched for the genome sequence of Bacillus stearothermophilus. This bacterium is thermophilic, meaning it can survive at extreme temperatures. They encountered a gene that is similar to genes encoding group II introns that have reverse transcriptase activity, and also provided supportive evidence for the 'RNA world' hypothesis. After the scientists cloned the gene and input into Escherichia coli to produce the protein, they confirmed it has reverse transcriptase activity and could operate at temperatures as high as 75oC. Dr Lampson and ETSU have now patented the reverse transcriptase and have licensed it to a company called InGex that developed it as a commercially available enzyme called TGIRT III.

Dr Lampson continues his research and lecturing at ETSU. His current research largely focusses upon looking at the metabolism of soil bacterium *Rhodococcus* and potential antibiotics produced by this bacterium. He also continues to take interest in reverse transcriptase and their diversity amongst bacteria. Collectively, his work shows that although bacteria can cause harmful, resistant infections, they also harbour proteins with useful applications that can be exploited and bring important benefits.

Meet the researcher

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Dr Bert Lampson received his PhD in Medical Microbiology from the University of Missouri-Columbia School of Medicine in 1986. He then undertook postdoctoral research in the laboratory of Dr Masayori Inouye at the University of Medicine and Dentistry, Robert Wood Johnson Medical School. In 1998, Dr Lampson became an Assistant Professor in the Department of Health Sciences at East Tennessee State University which was followed by promotion to his current role of Associate Professor in 2004. He has had a successful research career spanning four decades, during which time he has contributed significantly to tackling the worldwide problem that is antibiotic resistance.

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Professor Joseph T. Parisi (deceased)
Graduate students: J. Vellore, S. Moretz, T. Barber, M. Carr, R. Borisova, A. Ward and K. Sellick

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Department of Health Sciences, ETSU

EAST TENNESSEE STATE UNIVERSITY

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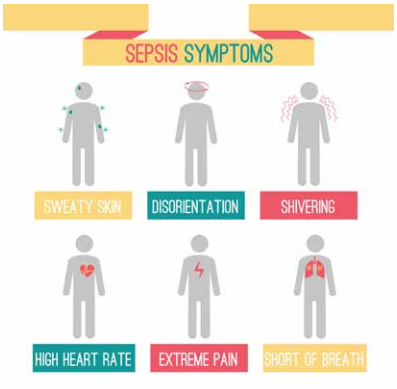
A FULLY INTEGRATED DIAGNOSTIC TEST TO DISCRIMINATE SEPSIS FROM INFECTION-NEGATIVE SYSTEMIC INFLAMMATION

Sepsis is a serious medical condition that manifests with a dysregulated immune response to an infection in the bloodstream, and is a major cause of morbidity and mortality worldwide. Many non-infectious conditions can lead to a state of hyper-inflammation known as the systemic inflammatory response syndrome (SIRS), which clinically can look very similar to sepsis. A lack of analytical tools that allow for the early detection of sepsis makes discrimination between sepsis and SIRS a very complex but critical diagnostic task. **Dr Richard B. Brandon**, **Dr Thomas D. Yager** and their colleagues at Immunexpress have developed a molecular diagnostic platform capable of detecting genetic biomarkers diagnostic for sepsis in about an hour. Early sepsis diagnosis can potentially lead to better patient management, more efficient use of resources, and a more sensible approach to the use of antibiotics.

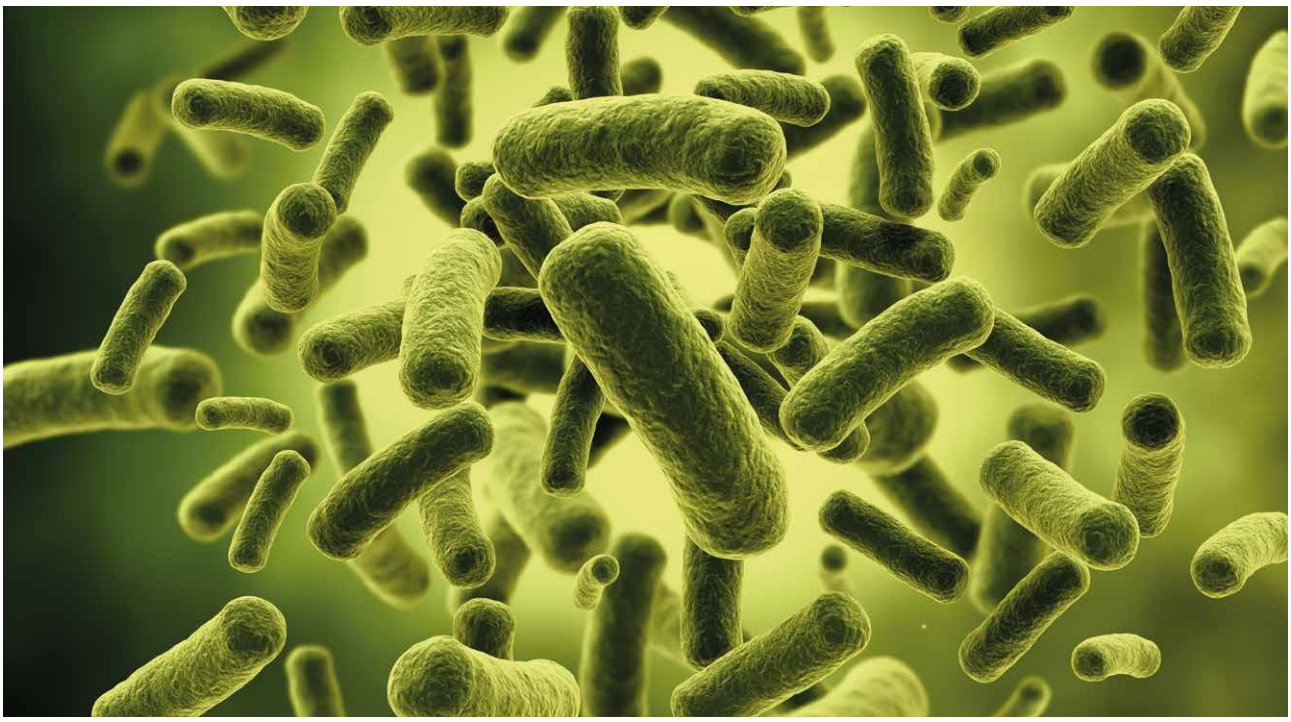
Differentiating Between Sepsis and Sterile Inflammation

During severe sepsis, the immune reaction becomes so dysregulated that multi-organ failure and death can result. It is important to note that not all patients presenting with a hyper-inflammatory response are septic. Some patients who have acute dehydration, intestinal or end organ ischemia, autoimmune disorders, adrenal insufficiency or haematological malignancy can also present with SIRS, a state of inflammation characterised by fever, increased heart rate and other clinical signs that are commonly associated with sepsis.

Although important, differentiating between sepsis and the non-infectious SIRS condition is a very challenging diagnostic task because patients present with similar clinical signs. The diagnostic tools currently available to most ICU physicians are less than adequate, leaving clinical staff with no choice but treat all patients suspected to have sepsis with antibiotics. However, the overuse of antibiotics can lead to side effects due to microbiome disruption, as well as the development of antibiotic-resistant infections among the most vulnerable patients. In any case, antibiotic therapy will not be effective in the treatment of viral sepsis.



Immunexpress is a molecular diagnostic company, founded in Australia and based in Seattle, Washington, USA, with the mission of improving the outcomes for patients suspected of sepsis. The Immunexpress team of researchers have discovered specific immune



system biomarkers, now implemented in SeptiCyte® technology that allows clinicians to diagnose sepsis accurately and rapidly, differentiating it from SIRS in critically ill patients.

The technology, which led initially to the development of a kit named SeptiCyte LAB, was recently transitioned to a next generation test, SeptiCyte RAPID, which is entirely self-contained in a cartridge that runs on the Biocartis Idylla™ system. Both tests rely on the same gene expression assay, but the newer version has a turnaround time for results of about an hour.

Importance of a Rapid Diagnosis of Sepsis

The timing of the diagnosis of sepsis can make the difference between survival and death. Physicians may or may not spot early sepsis signs in the initial stages of the disease, before multi-organ failure begins to occur and when treatment would be most effective. These early clinical signs can often be absent or indistinguishable from those of SIRS. The earlier sepsis is diagnosed, the greater are the chances of a favourable prognosis following therapeutic intervention.

Unfortunately, most of the current microbiology procedures adopted in the diagnosis of sepsis are affected by a large proportion of false positives (the wrong indication that a disease is present) or false negatives (the wrong indication that a disease is absent). In the absence of accurate and reliable diagnostic tools, physicians often rely on their clinical experience and intuition to make an early diagnosis of sepsis.

The variability in clinical presentations and in the training and experience of physicians make the diagnosis of sepsis particularly complex, especially before serious organ damage is evident. The team at Immunexpress quantified physician agreement in diagnosing SIRS or sepsis in a study published in 2019. They concluded in this work that there is a poor degree of consensus between different physicians in the differential diagnosis of sepsis. The least agreement was reached among physicians treating ICU patients presenting with clinical signs of respiratory illness.

Normally, sepsis is diagnosed through blood tests and cultures to confirm the presence of bacterial, viral or fungal infection along with other parameters, such as imbalanced electrolytes, reduced oxygen levels and increased levels of blood clotting factors. Unfortunately, blood tests that focus on confirming the presence of a pathogen take time to yield clinically actionable results, with most of these tests producing results in an about 24 to 48 hours.

A different approach to confirming infection, that potentially could yield results in about 1 hour, would be to look at molecular biomarkers that are up- or down-regulated when the immune system is responding to a pathogen. The human immune system responds specifically to an invading pathogen and expresses a variety of genes that produce detectable genetic signatures. These signatures could allow clinicians to discriminate between patients who are affected by sepsis from those who aren't.

The researchers at Immunexpress Inc. developed and validated a gene expression assay method based on the blood expression levels of four genes involved in the host response to infection. Their SeptiCyte LAB assay is able to detect the presence of sepsis biomarkers more rapidly – in approximately 6 hours – and more accurately than methods that rely on the direct identification of pathogens.

The SeptiCyte LAB test was cleared for market by the Food and Drug Administration (FDA) in the USA, on the basis of clinical trials involving 447 patients. The objective of the studies was to measure the performance of the assay method in discriminating between sepsis and SIRS in adults in critical care. Compared with other diagnostic methods, SeptiCyte LAB scored better at discriminating between cases of sepsis from cases of SIRS and the diagnostic power was not affected by the severity of disease. The method was confirmed to be both sensitive and specific in confirming the presence of infection, as it decreased the occurrence of false negative and false positive diagnoses compared to other diagnostic methods.

Development of a Near-patient Diagnostic Platform

Although cleared for market by the FDA, SeptiCyte LAB was never commercialised, due to its relatively long turnaround time (around 6 hours) in providing diagnostic evidence of sepsis. Its traditional workflow setup meant that testing had to be conducted in a centralised laboratory by highly experienced technicians.

To address these issues, the researchers at Immunexpress developed SeptiCyte RAPID, a sepsis diagnostic tool that relies on the measurement of two peripheral-blood gene-expression biomarkers using the same quantitative reverse transcription polymerase chain reaction (RT-qPCR) which highly correlates with SeptiCyte LAB clinical findings, but with a much easier workflow. Further clinical trials confirmed that SeptiCyte RAPID accurately differentiates between non-infectious systemic inflammation and sepsis.

The RAPID test is easy to administer and fully automated. The test requires minimal sample handling and can be performed using whole blood. The near-patient, cartridge-based RAPID test is fully integrated, from sample extraction, to sample analysis by RT-qPCR, and finally to the automated generation of a report. Since the technique does not rely on pathogen cultures, no incubation time is needed for the samples. In addition, samples are barcode-traceable. The RAPID platform provides results in around an hour, only requiring 2.5 ml of whole blood drawn into a PAXgene tube. The Idylla cartridge uses about 1/10 of the initial blood draw and only 2 minutes of set up time by a technician is required.

Although easier to use, the RAPID test correlates well with its manual counterpart in terms of accuracy of results. The technology can diagnose sepsis up to 48 hours prior to conventional microbiology-based methods, enabling physicians to choose the most appropriate treatment.

A further SeptiCyte product is being optimised that differentiates between bacterial and viral sepsis. This will be an important development, as viral sepsis does not respond to the antibiotic treatments routinely given to all patients suspected of presenting with sepsis. Early and accurate sepsis diagnosis in trauma and burns patients, and post-surgical patients suspected of infection, will lead to better patient management and outcomes, more efficient use of hospital resources, and more sensible use of antibiotics.

COVID-19 and the Management of Sepsis

Severe COVID-19 infections, like other viral infections, can lead to the sustained dysregulation of the host immune response that defines sepsis. Severe cases of COVID-19 can manifest with a wide range of clinical effects including breathing difficulties, hyperinflammation, confusion, accelerated heart rate, and even shock, multi-organ failure and death. Escalating clinical signs and symptoms that point toward multi-organ failure, taken together with a positive COVID-19 test result, confirms the presence of sepsis in severely ill patients.

The current COVID-19 crisis has highlighted the need for technologies that can discriminate between cases of mild infection from those that may develop sepsis. Sepsis is among the most common factors associated with COVID-19 mortality. The early diagnosis of bacterial and viral sepsis will assist in triaging COVID-19 patients, ensuring rapid sepsis management and saving lives.

Exciting Prospects for SeptiCyte RAPID

Early diagnosis of sepsis would lead to more appropriate treatment choices, improved prognosis, fewer days in intensive care and hospital, more efficient use of hospital resources and more appropriate use of antibiotic treatment, only when confirmed to be necessary.

Dr Brandon, Dr Yager and their colleagues at Immunexpress have successfully discovered, validated and patent protected Septicyte LAB and SeptiCyte RAPID. The latter is a fully-integrated, rapid, near-patient, gene expression diagnostic method that provides accurate results in 1 hour turnaround time to differentiate sepsis from SIRS. The team is now validating SeptiCyte RAPID in real-time studies across multiple sites in the USA and Europe and plans to launch the technology and get full FDA clearance by Q1 2021.

Meet the researchers

Dr Richard Bruce Brandon, PhD
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Dr Richard Bruce Brandon is a co-founder of Immunexpress and is currently the Chief Scientific Officer. Dr Brandon has a PhD in biochemistry and molecular genetics from the University of Queensland, Australia. His interest and knowledge of infectious disease, immunology, molecular genetics and veterinary clinical diagnostic procedures helped him develop the original concept behind the creation of Immunexpress, which was established in Australia in 2006 and has now expanded with offices in Seattle, USA.

Dr Thomas Dean Yager, PhD
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Dr Thomas Dean Yager obtained his PhD in Biochemistry and Biophysics from Oregon State University. In 2014, after several successful years as a consultant and lead scientist in the field of molecular diagnostics for various companies in Canada and the USA, he became Director of Research and Development at Immunexpress, where he had a fundamental role in developing the SeptiCyte RAPID cartridge test for discriminating between sepsis and SIRS.

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TARGETING THE IMMUNE SYSTEM TO OUR ADVANTAGE

The rise of pandemic-causing viruses is a worrying development arising as a by-product of our hugely connected world, and scientists must forge new paths to tackle these diseases. With researchers like **Dr Babita Agrawal** from the University of Alberta at the helm, we can hope to enter a new era of preventatives and therapeutics to help us fight disease. Dr Agrawal and her team are investigating novel vaccines and immunotherapeutics for influenza, Hepatitis C, tuberculosis, and even cancer.

Understanding Immunity

Immunity is a complicated phenomenon, encompassing the multitude of ways organisms can resist infection. Adaptive immunity is induced by both natural exposure to a pathogen and artificial exposure via vaccination, and it enables our bodies to become highly proficient disease-fighting machines. With the rise of antimicrobial resistance, novel vaccination strategies are an increasingly important way to optimise our own immunity and shift the focus from treatment to prevention.

Identifying the ways in which pathogens can trigger an immune response is a key element of vaccination strategies. This requires a strong understanding of the biochemical make-up of pathogens as well as of our immune system and how it becomes activated. With this knowledge, we can more effectively target viral diseases like influenza, Hepatitis C (HCV), and tuberculosis, which affect millions of people each year. Indeed, other global killers like cancer and autoimmune diseases can also benefit from our evolving knowledge of immunity as we develop therapeutics that can help when the immune system malfunctions.

Dr Agrawal and her team from the University of Alberta, Canada, are doing just that. Focussing on three specific projects, she hopes to improve our understanding of how the immune system can be targeted and harnessed to help us develop new therapeutics. These projects are investigating heterologous immunity and vaccination, subunit viral vaccines, and the role of T-cell modulation in chronic disease.

Heterologous Immunity

The concept of heterologous immunity is of particular interest to Dr Agrawal. This is the ability of a vaccine to induce immunity not just against its intended target but also against a pathogen for which it was not intended. The concept can be traced all the way back to the first vaccine discovered by Edward Jenner. He noticed that milkmaids were much less susceptible to smallpox because they were contracting cowpox, a virus in the same family. Over the course of his research, he was able to develop the cowpox virus into a vaccine which successfully conferred protection against smallpox as well.

The principal is fairly simple – protein sequences on the surface of one virus, like cowpox, engage our immune system to produce specific antibodies against it. Because the protein sequences on the surface of smallpox are similar enough, the antibodies against cowpox will recognise and attack the smallpox virus too. The antibodies are described as being cross-reactive. In addition to similarities between pathogens, T cells and antibodies in our body demonstrate significant plasticity in recognising their targets and contribute to heterologous immunity. The smallpox vaccine was developed to become more and more effective until the global vaccination program in the mid 20th century was able to achieve total eradication. Interestingly, scientists also observed a downward trend in other diseases like measles and scarlet fever.

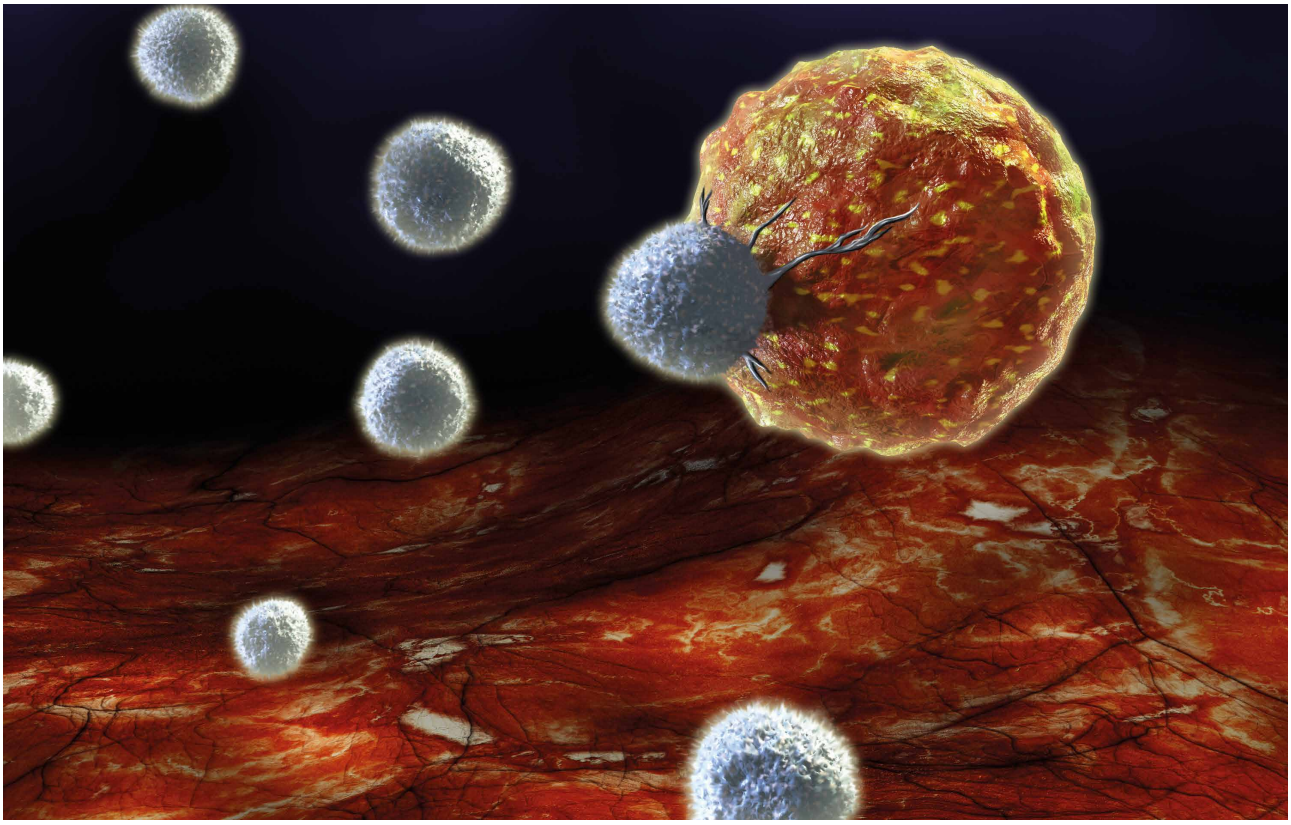


Illustration of T Cells

Dr Agrawal believes that the smallpox vaccine might have been inducing heterologous immunity against other pathogens. Physiologically, it would be desirable for our immune systems to be able to respond quickly to a wide range of pathogens, which might explain why antibodies and T cells can recognise more than just their intended target. Indeed, no human is immunologically naïve due to the near constant encounters with microbes both from our environment and within our own microbiome, giving us a library of immune cells which could cross-react with novel pathogens.

It remains difficult to accurately identify just what is at play when it comes to vaccine-related heterologous immunity. However, epidemiological studies of populations have revealed that it may be more prolific than we thought and could be aiding the prevention more than just the diseases people are vaccinated against.

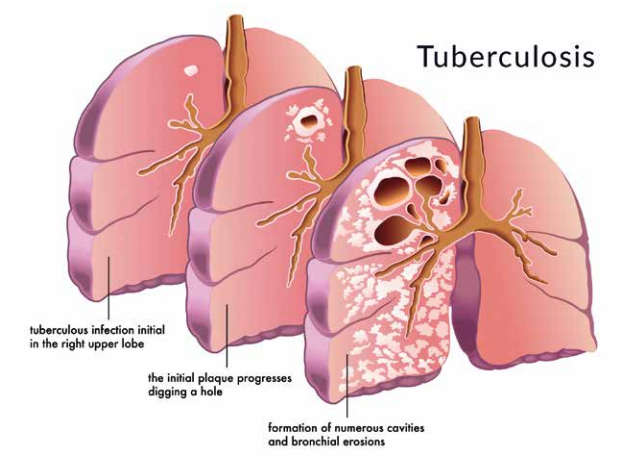
In a recent study, Dr Agrawal and her team identified similar protein sequences in the hepatitis C virus and adenovirus, suggesting the possibility of immune cross-reactivity. Their studies further demonstrated robust cross-reactive immunity between HCV and adenovirus. These original studies were recommended by F1000 Prime in 2016. By studying the mechanisms by which adenoviruses can induce a level of immunity to HCV she intends to find out whether this could be exploited clinically. Moreover, adenoviruses are a ubiquitous cold-causing pathogen and their high prevalence in our lives may have a subtle impact on HCV infection in populations. This is something Dr Agrawal is committed to studying further.

Adenoviruses are also a popular viral tool used by scientists due to the ease with which they can be modified which makes them unable to cause disease while maintaining their protein sequences. Because of this, they have widespread use as vectors for other vaccines and Dr Agrawal also considers the possibility that cross-reactivity induced by vaccination regimes may impact the prevalence of HCV infection as a by-product.

While heterologous immunity may be advantageous in increasing the repertoire of pathogens that we are protected against, the potentially harmful effects must also be considered before we try to make use of it. These include the possibility of antibodies cross-reacting with proteins on our own cells, causing autoimmune disorders, and a phenomenon known as antibody-dependent enhancement, where antibodies against a pathogen end up doing more harm than good. If we want to utilise the possible benefits of heterologous immunity, we must also identify the ways in which it could go wrong.

Fighting Microbial (or Mini) Giants

Dr Agrawal's second arm of research concerns the development of subunit vaccines for influenza and tuberculosis (TB). TB is incredibly difficult to treat due to its widespread drug resistance and eradication is hampered because



the only available vaccine, the Bacillus Calmette-Guérin (or BCG) vaccine is lacking in efficacy. Subunit vaccines take the protein sequences which trigger immunity and deliver them on their own, reducing side effects which come with using a whole-killed pathogen and being much easier to produce.

What Dr Agrawal and her team found was that a sequence within a TB protein called ESAT-6 was capable of engaging the immune system and enhancing its ability to respond to TB. This concept of 'boosting' the immune system is a common practice which is generally completed before vaccination to improve the immunity induced by it. Interestingly, this research demonstrated that administering the ESAT-6 sequence as a subunit vaccine after both BCG vaccination and TB infection was more effective in helping immune cells to clear it.

Dr Agrawal suggests that this may 're-educate' the protective immunity brought on by BCG vaccination. Currently, her team is exploring ways to broaden this immune response even more by incorporating other sequences from TB.

Building on her knowledge of viral proteins, Dr Agrawal wanted to go a step further and find out if the influenza virus possesses a common sequence between all strains which can be made highly immunogenic, allowing for a universal vaccine to be developed. Due to the ability of the influenza virus to constantly change the proteins it expresses on its surface, we currently implement seasonal vaccines which change each year, depending on which strain is most prevalent. Using a conserved sequence between all influenza strains would enable a single flu vaccine to be developed.

A Helping Hand

Dr Agrawal's final research focus is on how the immune system is regulated. Breakthroughs in immunology have revealed the essential role of T-cells in maintaining immune homeostasis and an effective immune response. To prevent autoimmunity, there are several checkpoints newly born T-cells need to pass which make sure that they are tolerant of the self and prevent ongoing tissue damage during inflammation. Researchers have identified modulator molecules, some of which act as stop signals, inhibiting the action of T-cells, and others which stimulate them.

The latter drugs have huge potential for application in cancer and some chronic infections, where the immune system's attempts to fight malignancy are thwarted. Stimulating the immune system with immunotherapy has been an important part of cancer therapeutics since its discovery, and in 1998 Dr Agrawal contributed significantly to the field with her discovery of a novel T-cell regulator, MUC1.

MUC1 is a protein known to be present on many tumour cells, but Dr Agrawal and her team made the serendipitous discovery that it is also present on activated T-cells. T-cells play many roles in immunity, including killing pathogens, and directing and controlling the immune response. By uncovering the ways that T-cells regulate the immune response we can better understand how it works and why it might go wrong.

Diseases like cancer as well as autoimmunity can be caused by a dysregulation of T-cell activity. Tumour cells are able to present inhibitory signals to T-cells, preventing them from doing their job. In autoimmunity, the tightly regulated checkpoints can fail, resulting in the generation of T-cells which mistakenly target and kill healthy cells. For both these conditions and many more, Dr Agrawal believes that MUC1 could be a valuable target for novel immunotherapeutics to restore the activity of T-cells to normal.

Dr Agrawal's research continues to focus on the interactions between the immune system and pathogens and how we can develop novel therapeutics to finetune this for our benefit. Looking to the future, Dr Agrawal hopes to lead the way in the development of novel vaccination strategies, preventatives, and immunotherapeutics for some of the world's most notorious diseases, with a particular focus on finding a universal influenza vaccine and a therapeutic vaccine for TB.

Meet the researcher

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Dr Babita Agrawal graduated from Allahabad University, India, in 1987 with an MSc In Biochemistry and went on to earn her PhD in Immunology from the University of Alberta, Canada, in 1993. She joined a major Canadian biotech company in 1996, receiving an award for outstanding achievement in research and development, before returning to the University of Alberta faculty as an Assistant Professor in 2001. Her widely acclaimed research focuses on immune regulation and novel vaccine strategies, paving the way for a better understanding of the immune system. She is the recipient of a Medical Scholar Award and a Senior Scholar Award from the Alberta Heritage Foundation for Medical Research (AHFMR), Dean's award from the Faculty of Medicine and Dentistry, University of Alberta, and has served as a scientific expert in grant panels for the National Institutes of Health (NIH), USA and Canadian Institutes of Health Research (CIHR).

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CURRENT FUNDING

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EXPLORING THE LINKS BETWEEN OXIDATIVE STRESS, RNA DAMAGE AND DISEASE

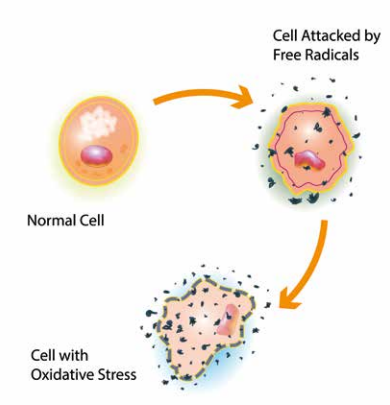
When the concentration of antioxidants and free radicals in your cells is out of balance, they experience oxidative stress. This may, in turn, result in damage to important cellular components that alter their original function, potentially having a role in the progression/development of disease. **Professor Marino Resendiz** from the University of Colorado Denver is researching how the modifications generated by oxidative stress alter the function and structure of RNA, an important component of all cellular organisms. His work has already demonstrated some of the changes that oxidative damage can result in, and how the oxidative modifications may potentially lead to novel structures with potential therapeutic uses.

RNA in Our Bodies

The importance of DNA and its role as genetic material is well known. However, you may not be quite as familiar with RNA, which stands for ribonucleic acid. Its structure is similar in many ways to that of DNA – they both contain units called nucleotides made up of a 5-carbon sugar, a phosphate group and a nitrogenous base. In DNA, these bases are adenine, cytosine, guanine and thymine, but in RNA the thymine is replaced with a base called uracil. Whereas DNA is found in the famous double helix structure, made up of two complementary strands (meaning the bases opposite each other always pair in the same way – cytosine with guanine and adenine with thymine), RNA is arguably more varied in terms of function, size, and molecular diversity.

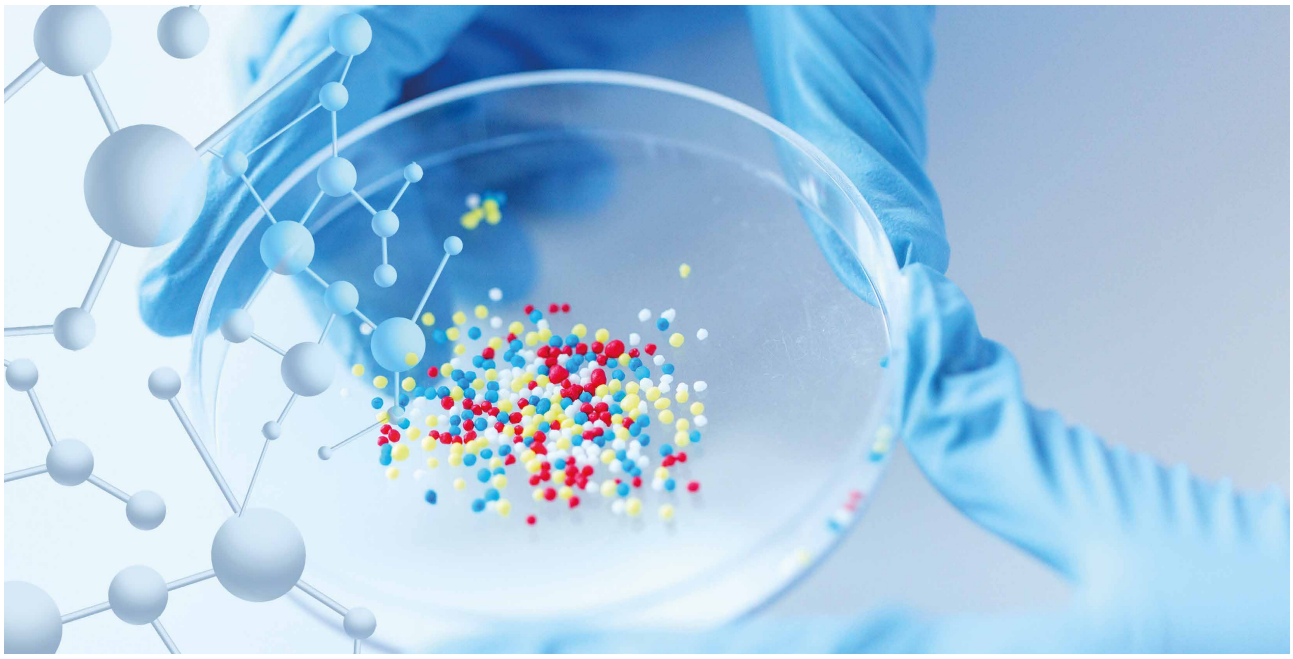
RNA is a macromolecule (or biopolymer), which means that it has a large structure made up of smaller units (nucleotides). In fact, both DNA and RNA are nucleic acids belonging to one of the four macromolecules that are essential for all known forms of life to function. The others are lipids, proteins and carbohydrates.

There exist different variations of RNA in cells, but each has a specific role in the processes of transcription and translation through which new proteins are created, or in the regulation of essential biological pathways. Some examples include messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). During transcription, an enzyme called RNA polymerase reads a section of DNA and produces a complementary strand of mRNA. After leaving the nucleus where it was made, the newly formed mRNA strand finds a ribosome which decodes it in segments. Bringing in



complementary tRNA fragments with amino acids bound to them allows the ribosome to piece together the amino acids and create a brand-new protein.

Through complex routes, RNA can control which genes are allowed to be turned into proteins (often referred to as 'expressed') as well as catalyse biological reactions, among other roles.



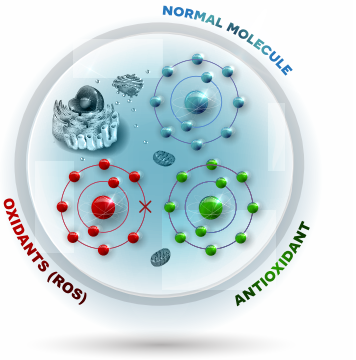
Oxidative Stress in RNA and Disease

One factor that can alter the structure of RNA, and hence its function, is an increase in the concentration of reactive oxygen species (ROS), some of which are free radicals. Because they are highly unstable, these molecules readily react with the biopolymers in the body, including RNA, which may cause problems for the health of a cell.

These unstable species are produced by the mitochondria as a result of metabolic activities, but they can also be generated in the body via other factors, including cigarette smoke, pesticides, and radiation, among others. Antioxidants produced by your cells usually counteract their effects, which can be enhanced or reduced by factors such as diet, pollution and radiation. An imbalance between reactive oxygen species and natural antioxidant processes is known as oxidative stress. Oxidative stress can be a positive thing – exercise causes an increase in free radicals whilst simultaneously increasing the production of antioxidants and facilitating tissue growth. However, if the body is exposed to oxidative stress over a long period of time, DNA, RNA and proteins can be damaged, leading to faster ageing and the potential for the development of a range of health issues.

Parkinson's disease and Alzheimer's disease are two examples in which progression is thought to be worsened by oxidative stress. Taking up about 20% of the body's oxygen supply, the brain performs extraordinary metabolic processes, presumably creating free radicals as they occur. This can aid with new brain cell growth and consequently, cognitive function. However, an accumulation may have severe consequences. If an excess of free radicals causes cell death, these neurological conditions may be triggered.

Understandably, finding out precisely how oxidative stress can cause disease is an important area of research for many scientists. Getting to grips with the mechanisms behind disease is an essential step in the development of therapeutics and preventative approaches. One such scientist is Professor Marino Resendiz at the University of Colorado Denver, who focusses his work on the changes oxidative stress creates in RNA molecules. Furthermore, he and his research group are taking advantage of the new interactions induced by oxidation processes and using them as tools to discover novel therapeutic tools.



Oxidative Stress Alters RNA: Structure and function

Professor Resendiz and his team understood that even though oxidative stress causes up to 25 times more damage to RNA than DNA, much less research had been done on the former. The resulting oxidative damage on an RNA strand is called an oxidative lesion, one of the most common of which arises from a guanine base to create a molecule called 8-oxo-7,8-dihydroguanine (8-oxoGua). The 'oxo' in that long name is due to the addition of the reactive oxygen species mentioned earlier. They aimed to find out how this lesion affects the structure and function of RNA and the consequence of these changes. More specifically, they wanted to understand how the presence of 8-oxoG induces changes in how RNA interacts with other important biomolecules.

Incorporating the 8-oxoGua lesion, using chemical procedures, into pieces of RNA has allowed the Resendiz laboratory to study if it altered its thermal stability (how well it stays together under heat) and whether its function changed, i.e., how it behaves in the presence of proteins or other small molecules. Stability was either increased or decreased depending on where the lesion was added, which may have interesting implications on the role that oxidative stress has on RNA, and its potential link to disease.

The position of the lesion also had an impact on the functionality of the RNA. Its affinity (how well it attracts certain molecules) was altered, potentially changing how it interacts with other structures and proteins within cells. Professor Resendiz and his team believe their findings from this study will be useful for future research into the effect oxidative stress has on RNA, how consequent pathways are modified, and how this may present as disease. In addition, while the presence of this lesion is typically seen as a negative factor, the Resendiz laboratory is taking advantage of its unique behaviour to build new RNA constructs with novel structural and functional properties with potential therapeutic applications.

Proteins and Damaged RNA – Ribonucleases

One group of proteins whose relationship with RNA might be changed after oxidative damage is known as ribonucleases. These enzymes are part of a family called nucleases, which cut up nucleic acids, like RNA, into smaller pieces. Ribonucleases are extremely important for maintaining 'healthy' RNA and if they are unable to recognise damaged strands, it may go unnoticed and cause the synthesis of faulty proteins. So, in a recent study, Professor Resendiz and his team set out to test RNA containing the same lesion as before (8-oxoGua) with three different ribonucleases.

Their interesting discoveries gave a novel insight into how damaged RNA is handled by these enzymes. One ribonuclease did not recognise the lesion at all, while another not only recognised but cleaved (cut) that area. This means that some damaged RNA will be degraded and put out of action, but others may not and go on to cause problems. Ongoing work in the Resendiz laboratory focuses on exploring these interactions with other ribonucleases, while aiming to understand their biological implications.

Proteins and Damaged RNA – Reverse Transcriptases

Certain viruses produce and utilise an enzyme called reverse transcriptase to replicate within their host. All of their genetic material is in the form of RNA, so they use reverse transcriptase to read their genes backwards, creating DNA to be inserted into the host's genome. In this way, the host unwittingly uses their own machinery to replicate and reproduce the virus that is infecting them. A couple of well-known examples of reverse-transcribing RNA viruses are hepatitis B and the human immunodeficiency virus (HIV).

Further work by Professor Resendiz investigated how oxidative damage and consequent lesions might respond to these viral enzymes. In addition to studying alterations caused by oxidative stress, they also looked into another common change that can happen to RNA in cells – the formation of inosine. When a sugar and adenine group (adenosine) lose an amine group, a new nucleotide called inosine is formed.

Professor Resendiz and his team outlined small molecular differences caused by these modifications that could have an impact on the way reverse transcriptase works on viral RNA and consequent infections. Their work will be useful as a reference for future studies into the topic, especially those studying viral biological pathways.

8-OxoG as a Novel Building Block in Aptamers

Aptamers are small pieces of RNA with a high affinity towards specific molecules. These occur naturally in biological systems and have been proposed as potential replacements of antibodies, and as powerful therapeutic agents and drugs. The current pieces used in the design of these constructs use nucleotides G, U, A, and C. The Resendiz team recently reported on the incorporation of 8-oxoG as a new building block to expand on the range for these constructs and to obtain aptamers with affinities towards different, biologically important, targets. They recognised that, while 8-oxoG can have a negative biological impact, it also offers distinct structural properties that can potentially be exploited in this context.

And There's More to Come

Professor Resendiz has made great progress extending our knowledge of how oxidative stress changes both the structure and the function of RNA. Better understanding these intriguing biological mechanisms of life and disease is important for future research and may even aid the development of therapeutics. Continuing his work at the University of Colorado Denver, Professor Resendiz will venture to delve even deeper into these processes.

Meet the researcher

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Professor Marino J. E. Resendiz graduated from the University of Utah in 2003 with a Bachelor of Science in chemistry. He went on to complete his PhD in organic chemistry at the University of California Los Angeles and then completed a postdoctoral position at John Hopkins University. Currently, Professor Resendiz works as a lecturer in organic chemistry at the University of Colorado in Denver, where he also maintains a highly active research profile. His research is focused on the relationship between the structure and function of RNA modified by oxidative stress and other chemical modifications. In particular, he looks at how this RNA interacts with small molecules and enzymes and investigates how his findings may be applied to real-world therapeutics.

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EMERGING APPROACHES TO THE DETECTION AND PREVENTION OF RHEUMATOID ARTHRITIS IN A PREDISPOSED INDIGENOUS NORTH AMERICAN POPULATION

Rheumatoid arthritis (RA) is a long-term, systematic disease that causes pain, swelling and stiffness in the joints. **Professor Hani El-Gabalawy** and his research team from the University of Manitoba in Canada are finding ways of better identifying those at risk for future RA as a prelude to developing and testing prevention strategies. Since other autoimmune diseases are known to have similar preclinical phases, prevention strategies that can effectively lower the risk of developing RA can potentially be adapted and applied to a broad range of similar disorders.

Prevalence and Impact of Rheumatoid Arthritis (RA)

Autoimmune diseases represent a heterogeneous group of chronic disorders affecting about 5% of the population and in which the immune system targets one's own body tissues causing progressive damage to tissues and organs. Rheumatoid arthritis (RA) is one of the most prevalent autoimmune diseases worldwide, and once established, it is typically a lifelong disease that can be suppressed effectively with medications but not cured.

Although RA is usually first diagnosed in clinical settings when individuals present with swollen and painful joints, it is now known that this is preceded by a prolonged preclinical phase where genetic and environmental factors interact to initiate the damaging autoimmune mechanisms that ultimately lead to disease. It is speculated that the detection of RA autoimmunity at this preclinical phase may afford opportunities for disease prevention and cure that cannot otherwise be achieved once RA is fully established.

Studies of RA in Indigenous North American (INA) Peoples

Many (but not all) Indigenous Peoples in North America exhibit disproportionately high rates of RA. A recent analysis from a large healthcare database in the Canadian Province of Manitoba showed that RA is 2–3 times more prevalent in Indigenous Peoples living in the Central Plains region (Cree, Ojibwa, Oji-Cree, Anicinabe and Dene), collectively known in Canada as First Nations (FN), compared to all other non-FN Manitobans. Furthermore, this FN population exhibits a much younger age of disease onset, frequent clustering of RA cases in families, and a tendency towards severe, rapidly disabling disease based on the frequent involvement of large joints such as the knees, elbows, shoulders, and hips. Thus, this common autoimmune disease is particularly devastating in the FN population, and understanding the biological basis of this is a key first step to improving the outcomes.

Gene-environment Interactions That Promote RA Autoantibody Development

The hallmark of many autoimmune diseases is the development of specific autoantibodies by the immune system that target normal self-antigens.

In targeting self-antigens, these rogue autoantibodies are capable of continually activating the full force of the body's inflammatory response, a powerful tool which is designed to rapidly and efficiently eliminate harmful bacteria and viruses. This inappropriate and unchecked autoimmune-mediated inflammation then causes irreversible damage to the cells, tissues, and organs that express their targeted self-antigen.

Autoimmune diseases become chronic and lifelong because the self-antigens, by their nature, always persist and cannot be eliminated the way that microbes are eliminated. Once the immune system develops a 'distaste' for a self-antigen and targets it, the response becomes exceedingly difficult to turn off and tends to amplify over time.

In RA, the most specific autoantibodies are anti-citrullinated protein antibodies (ACPA). The targets of these ACPA are proteins that contain the amino acid citrulline. Citrulline is generated in multiple proteins by a normal enzymatic reaction called citrullination where the amino acid arginine is slightly modified. Although the physiological roles of this common process continue to be explored, citrullination is an entirely normal biochemical reaction that occurs in everyone during inflammation and normal cell death. It can perhaps be thought of as part of the body's garbage collection system where specific proteins are marked for waste disposal.

So why do citrullinated proteins become the target of RA autoantibodies?

Multiple studies over the past 3 decades clearly point to specific interactions between an individual's genetic makeup and environmental factors. Both the genetic and environmental factors are quite common, and each alone is insufficient to generate RA autoimmunity.

More than one hundred different genes are associated with an increased risk of RA. Of these, the HLA genes are by far the most powerful predisposing genetic factors. HLA genes are responsible for making HLA proteins that are expressed on the surface of immune cells and regulate normal immune responses, particularly those of the adaptive immune system which generates immune memory. Individual variations in HLA genes are the reason why one individual can respond to a specific microbe in a highly effective manner while the next individual mounts a weaker and less effective response.

Specific variants of the HLA-DRB1 gene (and in turn, the surface proteins it encodes) are the primary risk factor for RA. There are several HLA-DRB1 variants known to be associated with RA in populations worldwide, most of which have a common amino acid sequence in their structure which has been termed the 'shared epitope' (SE). Individuals carrying HLA-DRB1 variants with this SE sequence are more likely to recognize and process autoantigens containing the amino acid citrulline in their sequence and then activate the immune system to respond to these proteins in an inflammatory manner.

Predisposed First Nations have a particularly high prevalence of HLA-DRB1*1402, an SE encoding allele that is essentially unique to the FN population. It is overrepresented in multiple Indigenous North American populations, all of which seem to have an increased risk of developing RA. The reasons why this HLA allele is so common in INA populations are intriguing and suggest that it may have afforded a survival advantage, possibly through enhanced immunity to particular infections, such as those brought to the New World from the Old World. Studies of skeletal remains before colonization suggest that RA may have been present in the Americas for thousands of years, whereas it did not appear in the Old World until after the Americas were colonized. Thus, HLA-DRB1*1402 may be the ultimate two-edged sword where increasing protecting from a deadly infectious disease are achieved at the expense of increased risk for developing the RA autoimmune disease.

To better understand the risk of RA development in FN People, and with uninterrupted funding from the Canadian Institutes of Health Research, in 2005 Professor Hani El-Gabalawy and his research team from the University of Manitoba established a prospective longitudinal cohort study of the close relatives of FN RA patients. This research methodology allows the study of individuals over time, and the aim here was to identify individuals at highest risk for developing future RA and follow them into disease onset. In turn, this would improve the understanding of how the autoimmune mechanisms that are established during the preclinical phase evolve and ultimately lead to the development of the highly destructive RA phenotype that is seen in this predisposed population. Fifteen years later, when the study participants who developed the earliest stages of RA were compared to the large number who did not, important insights were gained into how RA starts, and which autoimmune mechanisms need to be interrupted to potentially prevent onset.

Tools with Which to Study the Preclinical Phase of RA

Seminal retrospective studies from Sweden and the Netherlands showed that individuals who ultimately developed RA had detectable autoantibodies in their stored serum samples which had been obtained at the time of donating blood. Studies of stored serum samples available in a large repository obtained from USA military personnel have demonstrated similar findings, both for RA and the related autoimmune disease systemic lupus erythematosus (lupus). In all cases, the levels of the autoantibodies increased as disease onset approached, while the scope of self-antigens that were targeted by the autoantibodies expanded.

The majority of the known environmental risk factors for RA and lupus have been identified through case-control studies, in which diagnosed patients are directly compared with healthy individuals. Although this method has limitations because the data are collected primarily from the patient's recollection of exposure to environmental factors (and thus may not be entirely accurate), it is still very useful in exploring the association of an autoimmune rheumatic disease (ARD) with a specific risk factor. Notably, tobacco smoke has been identified as a strong risk factor for RA in several studies. However, whether smoking contributes to the initiation or propagation of RA still needs to be determined.

Predicting the Development of RA

Since ACPAs are a good biomarker for the imminent onset of RA, Professor El-Gabalawy's research team explored this further. The SE may contribute to an increased immune response against citrulline-expressing cells, but it does not explain the high levels of ACPAs in seemingly healthy individuals with no clinical evidence of the disease.

A study published in 2019 further refined the concept of RA biomarkers by characterising which ACPA molecules are associated with a higher risk of developing RA. It was confirmed that ACPA that feature additional sugar molecules in their structure called N-glycans are a particularly strong predictor of developing future RA. The ACPA, like all other antibodies, are produced by B lymphocytes and their offspring cells called plasma cells. In a normal immune system, the B lymphocytes go through consecutive cycles of maturation after being exposed to their target antigens to produce antibodies with a high affinity for the antigen so as to effectively eliminate bacteria and viruses. The addition of the N-glycans seems to be unique to the ACPA but does indicate that the B lymphocytes that produced these antibodies have undergone cycles of maturation, with the help of other lymphocytes called T helpers. Why this maturation occurs to produce antibodies again a self-antigen remains unclear and is an area of active research in several laboratories around the world.

Professor El-Gabalawy hypothesised that the detection of N-glycans in ACPA seropositive individuals could therefore serve as a better biomarker for future RA development and help improve the predictive capacity of the ACPA testing. Besides ACPA autoantibodies, the group also found multiple other proteins circulating in the blood that could further improve this predictive capacity and allow for targeting prevention treatments to individuals at highest risk for developing RA.

The Development of Inflammatory Arthritis (IA) in Relatives

RA in Indigenous Peoples tends to run in families, with a clustering of RA antibodies and inflammation markers found in first-degree relatives of RA patients. In a study published in 2019, Professor El-Gabalawy and his colleagues followed relatives with RA for 12 years and reported the link between the development of inflammatory arthritis (IA) and the prevalence of autoantibodies in FN families with RA. The study showed that relatives of patients with RA have an increased likelihood of being seropositive for ACPAs and rheumatoid factor (RF), and also for developing IA.

However, findings indicated that the prediction of RA based on autoantibodies should be approached cautiously, as these prospective observations indicated that a very large proportion of patients that were initially positive for either or both ACPA and RF tested negative after 5 years. Despite a high incidence of IA in the Indigenous (FN) families with RA, seropositive individuals did not necessarily develop IA and might even revert to a seronegative state, reflecting an absence of the antibodies found in seropositive patients.

Therapeutic Strategies and Limitations of the Detection of Autoantibodies

Although identifying at-risk populations is critical to disease prevention, commercial assays are progressively being developed that increase the sensitivity and specificity of the diagnosis of RA. Double seropositivity for both ACPAs and RF is associated with the highest probability of developing IA. Some therapeutic strategies aim to reduce specific circulating autoantibodies and there are many on-going clinical trials currently testing different drugs such as hydroxychloroquine for their disease modifying effects.

The likelihood of becoming seronegative, after showing the presence of detectable ACPA, suggests that the current understanding of RA based only on the presence of ACPAs and RF seropositivity is insufficient. Therefore, it is necessary to continue the study of the earlier phase of autoimmune disease, via prospective and longitudinal studies, to provide clinically actionable guidelines for ARDs. Professor El-Gabalawy and his team are hard at work to gather such prospective data on at-risk individuals to understand both the development and the evolution of this disease, and to develop biomarkers that improve both diagnosis and prognosis in ARDs such as RA.

Meet the researcher

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Professor Hani El-Gabalawy obtained his medical degree at the Faculty of Medicine at the University of Calgary in 1977, followed by his Internal Medicine training and Rheumatology Fellowship at Dalhousie and McGill Universities. In 1997, as a visiting scientist at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) in Bethesda, Maryland, Professor El-Gabalawy developed expertise in leading a large and complex research project focused on the early stages of inflammatory arthritis. In 2000, he returned to the University of Manitoba where he initiated a research program in the area of pathogenesis and prognosis of early inflammatory arthritis, with a particular focus on Indigenous North American populations that exhibit a disproportionately high burden of rheumatoid arthritis. From 2013–2017 Professor Hani El-Gabalawy served as the Scientific Director of the Canadian Institutes of Health Research (CIHR) Institute for Musculoskeletal Health and Arthritis. As a Professor of Medicine and Immunology, senior clinician-scientist, and Endowed Rheumatology Research Chair at the University of Manitoba, his research program continues to receive prestigious funding support for ongoing research in the prediction and prevention of rheumatoid arthritis in susceptible Indigenous North American People. Professor El-Gabalawy has received a large number of awards for his important contributions to the field of rheumatoid arthritis.

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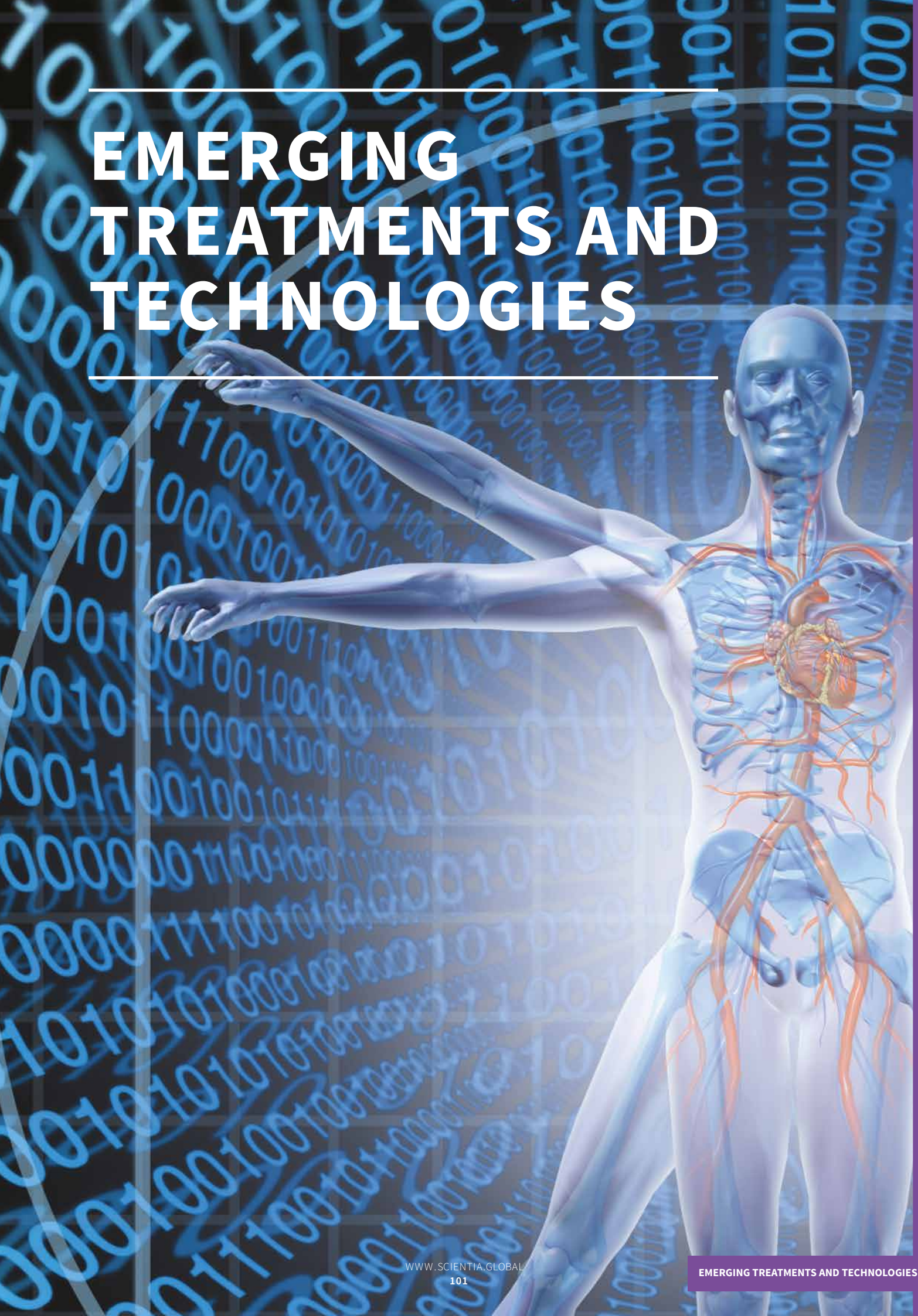
University of Manitoba

EMERGING TREATMENTS AND TECHNOLOGIES



EMERGING TREATMENTS AND TECHNOLOGIES IN HEALTH AND HEALTHCARE

From antibiotics to organ transplantation, there is no doubt that tremendous achievements have been made in healthcare. Yet still, there are many diseases and disorders that remain significant threats to health and even life itself in the current day. Heart disease and cancer, for example, remain global killers and at the time of writing, we are still battling the COVID-19 pandemic across the world. But we know that research saves lives. Our final section of this issue celebrates the work of scientists who are dedicated to the development of innovative treatments and technologies, and in doing so, are shaping the healthcare of the future.

We open this section by meeting Dr Iain Buxton at the University of Nevada. The sadly common but potentially devastating occurrence of preterm (premature) birth is not yet preventable but Dr Buxton is determined to change that. We read how he has revolutionised understanding of the role of smooth muscle of the uterus in preterm birth by challenging the status quo in this field. His important work is paving the way for novel treatments for preterm labour and birth that hold the potential to save the lives of many newborns.

Skin squamous cell carcinoma is one of the most common forms of cancer and new, innovative treatments are urgently required. Dr John T. Seykora at the University of Philadelphia is at the forefront of research in this critical area. We read how he has developed a highly promising targeted therapy in which pre-cancerous skin cells are treated with a topical cream to reduce the number of harmful cells and potentially inhibit the growth of the cancer at this early stage.

We then turn to the field of vision science. Dr Zi-Bing Jin from the Beijing Institute of Ophthalmology is conducting several lines of research with the aim of overcoming leading causes of sight loss, such as damage and disease of the cornea. We read how Dr Jin has discovered a new method for creating the necessary cells for corneal transplant, which critically, does not require a cornea donor. Another cause of vision loss is driven by mutations affecting the expression of cone photoreceptors in the retina. Here, we read how Dr Jin is pioneering the development of preclinical models that offer important opportunities for studies on vision disease mechanisms as well as therapeutic development.

Professor Gemmy Cheung holds senior roles at the Singapore National Eye Centre and the Singapore Eye Research Institute. Dr Cheung has brought together a group of expert scientists to form the Translational Asian Age-Related Macular Degeneration Program. Their focus is on ascertaining the underlying mechanisms of age-related macular degeneration which they are using to inform the development of novel therapies, improve diagnosis and develop tools to better understand the impact of the disease.

Across the world, heart disease is a leading cause of fatality. After injury, the heart cannot regenerate, and over time this can lead to heart disease. Dr Claudine Bruck at Prolifagen, Professor Edward Morrisey and Professor Jason Burdick (both at the University of Pennsylvania), have collaborated to develop a novel therapy to regenerate damaged heart muscle. We read how this novel therapy has the potential to improve the outcomes for millions of patients following a heart attack.

Professor Wilfred D. Stein takes the novel approach of applying mathematics to biology to better understand the elusive details of life. In collaboration with his colleagues at the Hebrew University of Jerusalem and institutes worldwide, he has progressed the use of quantitative tools to tackle diverse topics ranging from cancer treatment to drug-resistant malaria. We read how his work has supported scientists in many different areas of biomedicine.

Two collaborative research groups based in Germany and funded by the German Research Foundation are the next focus in this section. Collaborative Research Centre 1340 is based at the Charité – Universitätsmedizin Berlin and represents a large collaboration of experts in biochemistry, physics and medicine. We read how this multidisciplinary approach is establishing new and innovative methods for medical imaging and research at the anatomical and molecular levels.

Collaborative Research Centre 1309 is led by Professor Thomas Carell at the Ludwig-Maximilians University. This large collaboration of researchers across Germany aims to elucidate the chemical biology of the second layer of information that lies beyond the genetic sequence. We read how this layer of information is responsible for encoding epigenetic modification, that is, changes made by behaviours and the environment that can affect how genes work.

We then turn to the work of Dr Guo-Qiang Zhang at the University of Texas Health Science Center. In modern healthcare, data that are generated and shared are growing in volume and complexity at a phenomenal pace, and unsurprisingly, traditional approaches to handling such large masses of information are often inadequate. Dr Zhang has made it his mission to develop user-friendly query engines that simplify the process of clinical data management. We read how his work is improving clinical care and practice in a diverse range of fields, including cancer, epilepsy and sleep research.

We conclude this section by meeting Dr Michael Schutz from McMaster University. Incessant 'beeps' from medical devices are a routine sound in hospital environments, with functions including the monitoring of patient vital signs and alerting clinicians to imminent emergencies. Despite these critical functions, incessant beeps can also create problems for clinicians and patients, for example, by being distracting, confusing or annoying. We read how Dr Schutz takes the novel approach of applying his expertise and insight about the use of sound by musicians to improve the quality of the auditory signals of life-saving devices used across the world.

REVOLUTIONISING UNDERSTANDING OF THE MYOMETRIUM TO PREVENT PRETERM BIRTH

Great advances in the field of science and medicine have seen treatments and cures developed for some of the worst diseases ever known. Despite this, the potentially devastating, but sadly common occurrence of preterm birth is not yet preventable. **Dr Iain Buxton** at the University of Nevada, USA, has been studying the role of the smooth muscle of the uterus to elucidate its role in preterm labour and birth. His research is paving the way for the development of much-needed interventions to prevent early birth.

Preterm Birth: A Devastating Problem That Is on the Rise

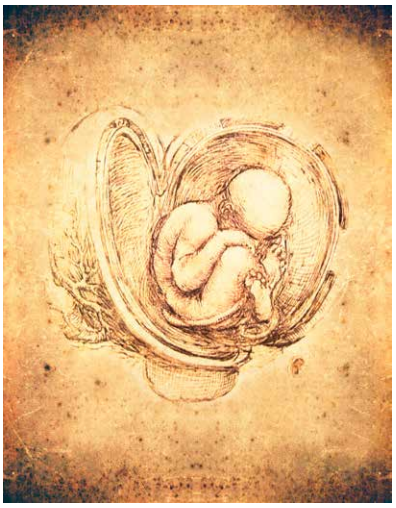
Preterm birth is defined as the birth of a baby before 37 weeks of completed gestation. According to World Health Organization statistics, an estimated 15 million babies annually are born prematurely. Approximately 1 million children die each year as a consequence of preterm birth and those who survive are at risk of lifelong disabilities. As such, preterm birth is the leading cause of death in children aged under 5 years, and an estimated 75% of paediatric care is spent on children who are born preterm.

The most alarming statistic of all is that the prevalence of preterm birth is rising. And as of yet, there is no Food and Drug Administration approved medication to prevent preterm labour. Dr Iain Buxton at the University of Nevada, USA, believes that understanding how the smooth muscle of the uterus contracts and, particularly how it relaxes, could be critical in helping prevent preterm labour and birth.

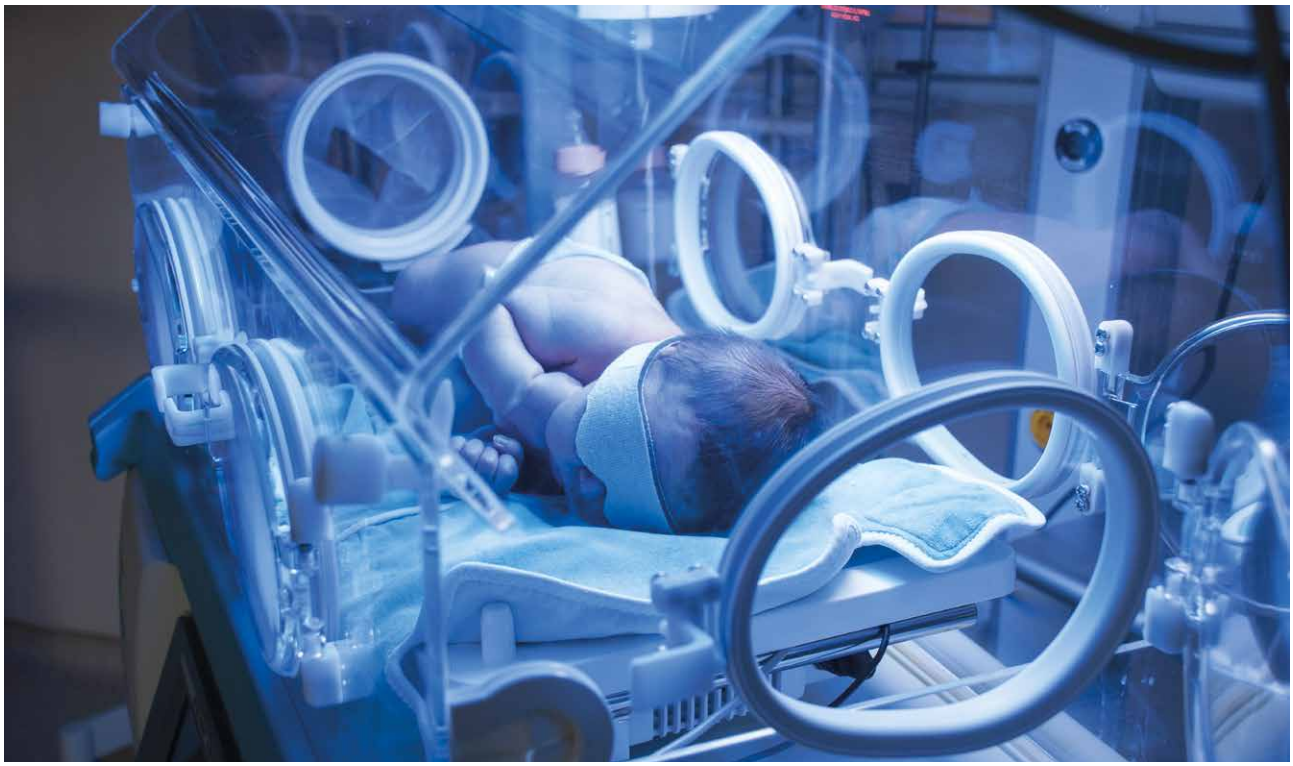
Dr Buxton had previously worked with Laurence Brunton, a student of Dr Alfred G. Gilman, the pioneer in pharmacology and biochemistry who was awarded a Nobel Prize along with Dr Martin Rodbell in 1994 for the discovery of G-proteins, the proteins essential for signal transduction in cells. Dr Buxton had great expectations from both himself and others to succeed as he branched out on his own to continue research in Reno.

The Nobel Dogma

The myometrium is the middle layer of the inner wall of the uterus. It consists of smooth muscle and its basic function is to induce contractions. Blood vessels are also lined with smooth muscle, and so, researchers have studied blood vessels as a means to understand smooth muscle functionality. Much of this work, carried out in animal models, has been conducted on the aorta, which is the large blood vessel that carries blood from the heart to the rest of the body.



Alfred Nobel.

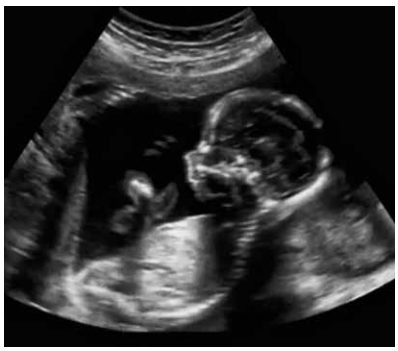


Early work determined that endothelial cells that line the blood vessel release a substance called endothelial dependent relaxing factor that acts on the smooth muscle cells. This factor was later identified to be nitric oxide (NO) and its role in the smooth muscle activation pathway was determined. Researchers described how NO released from endothelial cells acts on underlying smooth muscle cells to activate an enzyme called guanylyl cyclase, which in turn generates cyclic guanosine monophosphate (cGMP).

cGMP acts as a messenger in the muscle by partnering with a kinase that adds a phosphate group to myosin phosphatase, activating it, which then relaxes the muscle. In 1998, Dr Robert Furchgott and Dr Louis Ignaro from the University of California, Los Angeles, along with Dr Ferid Murad from the University of Virginia, received a Nobel Prize for this pivotal discovery. Dr Buxton hypothesised that the action of NO relaxes the smooth muscle of the uterus. Initially, he had reasoned that NO must act upon the uterus as it does on blood vessels and other smooth muscles – but he soon realised this was not the case.

A Biochemical Conundrum

Early studies by Dr Buxton's laboratory in guinea pigs showed that although in blood vessels NO acts on the smooth muscle through guanylyl cyclase activation and cGMP accumulation (causing it to relax), in the guinea pig myometrium NO has no effect on spontaneous contraction, but does relax oxytocin and acetylcholine-evoked contractions. This is associated with significant elevations in intracellular cGMP. However, on inhibition of guanylyl cyclase, cGMP accumulation is blocked, yet NO is still able to relax contractions caused by oxytocin or acetylcholine.
These studies suggested that contractions in the pregnant and non-pregnant guinea pig uterus occur in a manner that is not guanylyl cyclase dependent. Dr Buxton's laboratory found the same to be true in monkey and human myometrium tissue. At the time, these findings were considered heresy since they contradicted what scientists then knew about the function of smooth muscle. Dr Buxton and his colleagues sought to solve this conundrum by going back and studying the very basics of myometrial relaxation. The mechanisms now established for cGMP independent NO signalling involve the S-nitrosation of proteins. Dr Buxton explains 'target proteins are nitrosated…an amino acid in their sequence, cysteine, is chemically modified with the addition of a NO moiety. The resulting protein is said to be nitrosated.' There was evidence that S-nitrosation may be involved in calcium release which may activate potassium channels; however, this pathway had not been explored in the myometrium. Attention then turned to investigating calcium activated potassium channels as a possible way in which NO relaxes the myometrium.
The group hypothesised that cGMP action in the myometrium is compartmented. A published paper of the findings describes how cGMP may be regulating the relaxation of uterine smooth muscle. Uroguanylin is an activator of guanylyl cyclase and relaxes oxytocin-induced contractions in pregnant guinea pig myometrium. The team found that after uroguanylin stimulation, relaxation and cGMP accumulation is blocked by guanylyl cyclase inhibitors. The overall results of



this study led the group to conclude that a uroguanylin cyclase-cGMP relaxation pathway is present in myometrium smooth muscle and the cGMP is compartmented in the myometrium such that other agonists that elevate cGMP do not result in relaxation.

The Disparate Pathway in Myometrium

Dr Buxton's work slowly became accepted such that he now describes it as 'the most important exception to Nobel dogma because it lays out a fundamental distinction about the human myometrium.' The team then wanted to investigate if stretch-activated, two-pore potassium channels (K2P) are gestationally regulated in human myometrium and whether they contribute to uterine relaxation. TREK-1, TREK-2 and TRAAK belong to the TRAAK family subset of K2P channels. Dr Buxton's laboratory decided to investigate the expression and differential regulation of TRAAK family channels in pregnant and labouring human myometrium.

In a 2010 paper documenting the results, Dr Buxton describes the importance of the findings to the development of potential therapeutic targets in preterm labour. The results showed that the TREK-1 potassium channel is expressed and up-regulated substantially during pregnancy. In labouring tissue, TREK-1 is down-regulated, indicating a role of TREK-1 in uterine quiescence prior to labour since the uterus expands and stretches during gestation.

It was now clear that NO relaxes uterine smooth muscle but not in the way it does in other smooth muscles. The evidence from Dr Buxton's group, as well as that from other researchers, converges to suggest that S-nitrosation is the most likely mechanism by which NO relaxes the myometrium.

Dr Buxton and his colleagues described the myometrial S-nitrosylproteome (i.e., the entire set of S-nitrosated proteins that are expressed or can be expressed by the tissue) in pregnant and non-pregnant guinea pig tissue. This was the first attempt to examine the S-nitrosylproteome of the myometrium.

The findings revealed that specific groups of proteins that are involved in contraction and relaxation are S-nitrosated in labour and gestational muscle. Many of the proteins were found to be nitrosated in only the pregnant state or only the non-pregnant state and the team further describe how S-nitrosation of a filament protein called desmin is increased more than five-fold in pregnancy.
Moreover, these studies identified many proteins important in the mechanisms that regulate uterine smooth muscle function and with better understanding, such proteins may prove to be new tocolytic (anti-contraction/labour preventing) targets to prevent the possible dysfunction of smooth muscle which may lead to preterm labour and birth.

New Avenues for Preventing Preterm Labour

S-nitrosoglutathione reductase is the enzymatic regulator of endogenous S-nitrosoglutathione (involved in NO signalling and is a source of bioavailable NO). More recently, Dr Scott Barnett, a core member of Buxton's research team found that this reductase is upregulated in preterm myometrium and is associated with reduced total protein S-nitrosation. They observed that on blocking S-nitrosoglutathione reductase, the smooth muscle of spontaneous preterm labour tissue is relaxed. In the paper documenting these findings, Dr Buxton's team describe that the 'failure of guanylyl cyclase activation to mediate relaxation in spontaneous preterm labour tissues, together with the ability of NO to relax term labour, but not spontaneous preterm labour myometrium, suggests a unique pathway for NO-mediated relaxation in myometrium.'

To further explore the function of S-nitrosation regulation in maintaining uterine quiescence, the Buxton laboratory studied nebivolol. Nebivolol is primarily a ß-blocker (a class of drug to treat abnormal heart rhythm), but it is unique because it also has the ability to relax the smooth muscle in the walls of blood vessels (vasodilation) and does so in part by potentiating the action of NO. The researchers found that in uterine smooth muscle from mice and humans, oxytocin-induced contractions are relaxed by nebivolol. The researchers explain one of the advantages of using nebivolol as a tocolytic is that it is already FDA-approved for use as a ß-blocker. However, more studies are required to assess the safety and efficacy of this drug in pregnant women.

Dr Buxton's important work over the years has determined the fundamental physiological distinction in tissues from women who deliver preterm. Dr Buxton tells us that 'a complete understanding of the underlying biochemical difference at the root of this distinction can lead to defining a target for drug development.' He further shares with us how his team is currently collaborating with ExCyte Therapeutics to develop new tocolytic mediations based on the novel S-nitrosation pathway that they have described. Addressing the problem with dual ligand drugs that can interact with more than one pathway while avoiding the unwanted effects of single agents employed at higher doses is currently underway under the supervision of Dr Barnett. This research is now paving the way for promising treatments for preterm labour and birth with the potential to save many newborn lives.

Meet the researcher

Emerald Bay. Credit Iain Buxton.

Dr Scott Barnett

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Dr Iain Buxton received PhD training in Protein Chemistry and Enzymology from North Carolina State University and his Doctor of Pharmacy in Pharmaceutical Sciences from the University of the Pacific, USA. Born in Buckinghamshire, England and raised in California, USA, Dr Buxton presented his first research paper at age 19. Dr Buxton completed his clinical residency at the Veterans Affairs Medical Centre in La Jolla and his postdoctoral studies in the Department of Medicine at the University of California, San Diego School of Medicine. Currently Foundation Professor in the Department of Pharmacology at the University of Nevada, Reno School of Medicine, Dr Buxton's laboratory focuses on nitric oxide signalling in smooth muscle and preterm labour. Buxton has also contributed to understanding cancer cell dormancy and metastasis in breast cancer. In addition, he has been a member of numerous medical boards and an editorial board member of many research journals. He is also the founder and CEO of ExCyte Therapeutics.

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FUNDING

National Institutes of Health
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PROMISING NEW TARGETED THERAPIES IN THE TREATMENT OF SKIN CANCER

Every year, approximately 700,000 new diagnoses of squamous cell carcinoma, a type of skin cancer, are reported to dermatologists in the USA alone, constituting the second most common form of cancer. Treating pre-cancerous skin cells with a therapeutic topical cream could significantly reduce the number of harmful cells, potentially inhibiting the growth of squamous cell carcinoma. **Dr John T. Seykora** and his team from the University of Philadelphia have been researching the biology of skin squamous cell carcinoma and related precursor lesions to identify new therapeutic targets that could be treated using topical approaches with exciting results.

Squamous Cell Carcinoma: A Growing Problem

In our ageing yet active population, skin cancers such as squamous cell carcinoma and associated precancerous lesions are becoming increasingly common, and the need for new therapeutic strategies becomes more pressing by the day. Squamous cell carcinoma *in situ* is a common pre-cancerous skin condition that can progress to the more serious disease of cutaneous squamous cell carcinoma. Although the former is frequently seen by dermatologists, our understanding of the biological mechanisms behind the condition remains incomplete.

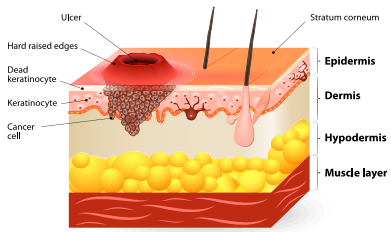
Understanding human skin cancer development requires scientific studies that elucidate the molecular pathways leading to pre-cancerous lesion formation and subsequent tumour development and disease progression. As such, laboratory mice have proven to be invaluable resources as models in this work. Currently, Dr John T. Seykora and his team from the University of Philadelphia are leading the scientific effort through their experiments on genetically engineered murine models and in-depth characterisation of human samples.

Dr Seykora has observed the clinical and molecular factors relating to skin cancers to define the progression from unremarkable epidermis to *in situ* lesions to cutaneous squamous cell carcinoma by utilising a variety of experimental approaches. Furthermore, insights gained from computer modelling of these lesions have identified multiple targets for treating pre-cancerous lesions.

The Development of Cancer

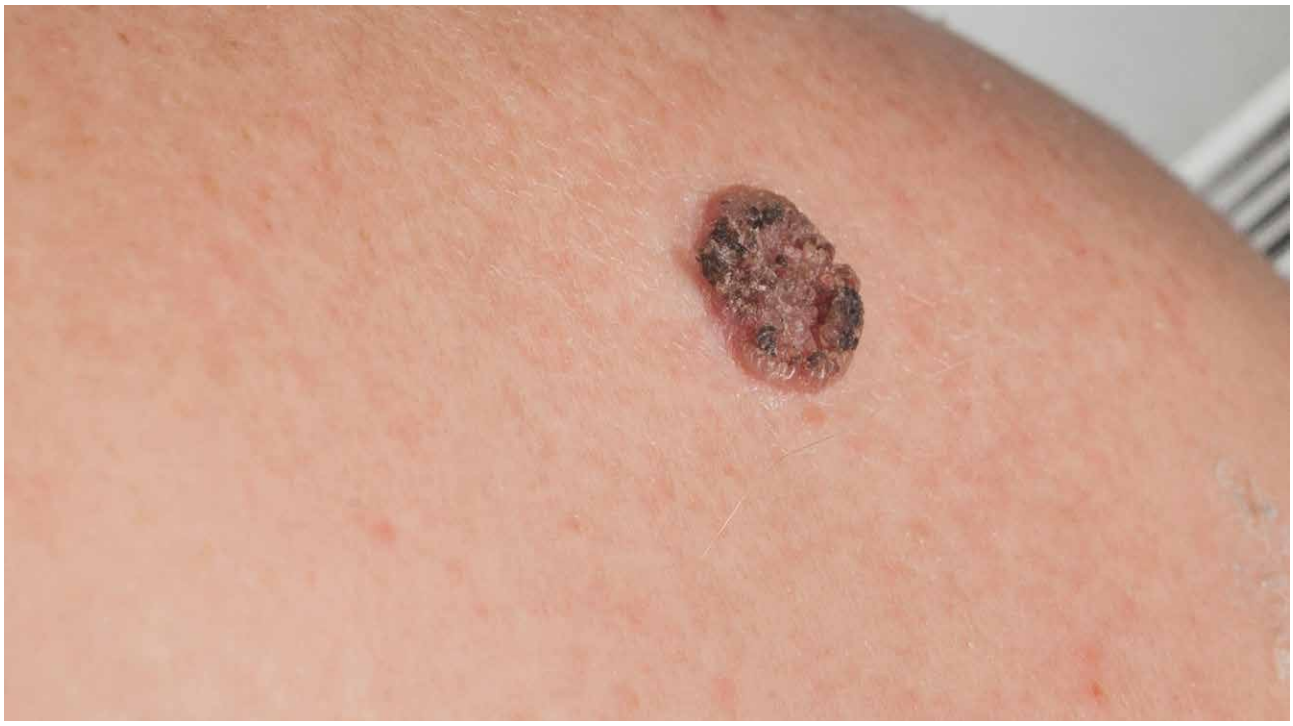
As is the case with other cancers, cutaneous squamous cell carcinomas exhibit a high rate of DNA mutations. The mechanisms that lead to this high frequency of DNA mutations likely result from tumour-suppressor proteins and proto-oncogenes being damaged via exposure to ultraviolet (UV) light. The UV-induced DNA mutations responsible for producing the *in situ* lesions in human skin were demonstrated in a 2021 study by Dr Seykora's group on human skin specimens.

Squamous-cell carcinoma



Since these initial observations of ultraviolet-induced protein mutations, other studies have confirmed the presence of mutations in significant numbers of squamous cell carcinoma patients. Previous studies by other researchers had presented a high prevalence of anti-cancer mutations in sun-exposed skin in comparison to non–sun-exposed skin, setting the stage for Dr Seykora's own studies in this field. Consistent with these findings, Dr Seykora's team found that 60% of patients displaying pre-cancerous

'These innovations represent a promising and much-needed alternative to today's topical therapies, one that directly targets the biological mechanisms driving the tumour's growth…'



squamous cell carcinoma *in situ* had similar anti-cancer protein mutations, indicating that the loss in these useful proteins in skin cells occurs before full cancer can develop.

Although laboratory mice are a strong, versatile model for studying skin cancer development, the key differences in skin structure between mice and humans limit any direct correlations between mice-based skin cancer studies and human disease. Additionally, there are major differences in both biochemical and genetic factors. Therefore, the correlation of observations made in mice models with those present in human skin tissue is vital for demonstrating relevance to human disease.

Multiple epidemiologic and basic research studies, including a 2019 study by Dr Seykora and his research team, have shown that cutaneous squamous cell carcinoma can also come about via chronic exposure to a systemic anti-fungal agent called Voriconazole, especially if the patient's skin has been extensively exposed to UV radiation.

These observations suggest that Voriconazole promotes some sort of biochemical reaction in skin cells that makes them more vulnerable to UV irradiation, ultimately leading to cancerous tumour formation. Since these antifungal treatments have been found to produce cutaneous squamous cell carcinomas in UV-exposed skin, these compounds likely promote additional DNA and cellular damage provided by UV radiation to accelerate the formation of tumours. The mechanism responsible for this process, however, was unclear until Dr Seykora's group showed that Voriconazole directly inhibits the enzyme catalase which is a key detoxifying protein in cells that limits UV-induced damage and DNA mutations.

Research into the basic molecular mechanisms of skin cancers is the foundation for testing new therapies that are critical in combatting the disease. Back in 2002, Dr Seykora and his research team discovered that a novel cancer-preventing protein acts as a vital marker in understanding whether or not skin cells may be potentially predisposed to developing skin cancer. These studies identified a class of anti-oncogenes that target activated tyrosine kinases for degradation; this protein was named Src Activating and Signaling Molecule (Srcasm). Srcasm levels are inversely proportional to the rate of cell proliferation in cSCC and *in situ* lesions and Src tyrosine kinases, the target of Srcasm, are therapeutic targets for cSCC and related precancerous lesions. Therefore, molecules that enhance Srcasm function also may represent a new class of therapeutic agents to treat these lesions.

Future Potential Treatment Options

Current treatments methods for *in situ* and cutaneous squamous cell carcinomas are limited and invasive. Surgery is often recommended to remove squamous cell carcinomas, particularly those classified as high risk. Surgical removal involves injecting a local anaesthetic and removing the tumour from the skin along with a clear 'safety margin' to ensure that all of the cancer cells have been removed.

Moh's surgery is a commonly used technique, involving the surgeon removing skin tissue layer by layer, mapping and freezing each layer, and examining the tissue for tumour cells under a microscope before proceeding to the next layer. This method is complicated and time-consuming but ensures that the entire tumour is removed with as little scarring as possible. Other options include radiation therapy, which takes a long time and can be painful, and other surgical approaches. A treatment method that does not cause pain or irritation, is non-invasive as well as cost-effective, could prove hugely beneficial for patients suffering from this type of skin cancer.

Since cutaneous squamous cell carcinomas typically arise from less-developed squamous cell carcinomas *in situ*, effective treatment of these affected 'precursor' skin lesions could potentially decrease the number of fully-developed cancer patients. Current treatments for these precursor skin conditions include cryotherapy (freezing the skin) or minor surgery, which can lead to skin pigmentation and scarring – as well as not being cost-effective. Currently available topical therapies for this stage include Imiquimod and Fluorouracil creams.

Fluorouracil and Imiquimod produce irritating, often painful inflammation, which discourages patients from completing the full course of treatment, although other topical treatment options are proven to have suboptimal pre-cancerous cell reduction rates or are very expensive for the patient or medical service provider. There remains a clear medical need for effective topical therapies that lack these serious and adverse side effects.

In 2008, Dr Seykora's group published that Src-family tyrosine kinases are hyperactivated in human actinic keratosis, *in situ* carcinoma and squamous cell carcinoma. This study indicated that Src kinases are a therapeutic target for treating these lesions. Based on these studies, Dr Seykora's group derived in vivo murine models of these lesions and tested the potential utility of topically applied kinase inhibitors to treat cutaneous squamous cell carcinomas and related precursor lesions.

A recent study of the kinase inhibitor Dasatinib shows that topical application promoted shrinkage of cancerous tumours in a similar way to topical Fluorouracil cream, but caused less painful inflammation and produced no skin ulcers. Dasatinib was applied daily to experimental mice, and was found to cause 45% and 77% reduction of cutaneous squamous cell carcinoma cells after two to five weeks of treatment, respectively.

Fluorouracil cream was also tested in this study and was found to induce a 70% regression in skin cancer cells after two weeks. However, it was found to cause skin ulcers in two out of the 15 observed tumours, and seven of the eight mice in that group died. No ulcers or deaths were observed in the Dasatinib topical treatment group. Dr Seykora, when asked in a recent interview, stated that 'Together, these data suggest that topical application of small-molecule kinase inhibitors may be useful for treating cutaneous squamous cell carcinoma and related precursor lesions.'

In parallel experiments that were also undertaken by Dr Seykora's research team, another topical skin cancer treatment that works in a similar way to Dasatinib, known as Dactolisib, was found to reduce the size of cutaneous squamous cell carcinomas in comparison to the currently available treatments.

Taken together, these results suggest that the topical application of new treatments targeting the proteins associated with cancer cell formation have the potential to treat cutaneous squamous cell carcinomas and other related skin conditions, and raise the possibility that topical treatments containing multiple cancer-associated protein (enzyme) inhibitors together could be more effective than utilising a single compound.

'These innovations represent a promising and much-needed alternative to today's topical therapies, one that directly targets the biological mechanisms driving the tumour's growth,' explains Dr Seykora, 'with the number of cases of cutaneous squamous cell carcinoma steadily rising, it's important we work to identify safer, more precise therapies to treat these cancers before they progress and spread. We believe topical kinase inhibitors are potential candidates that warrant further study.'

Meet the researcher

Dr John T. Seykora
Perelman School of Medicine
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Dr John T. Seykora is a tenured Professor of Dermatology in the Perelman School of Medicine at the University of Pennsylvania. He received his undergraduate degree in Biology with Honors and concurrently was awarded a Masters in Biochemistry from the University of Chicago. Dr Seykora received his PhD from Rockefeller University, and then an MD qualification from Cornell University Medical College. After undertaking residency and fellowship at the University of Pennsylvania, Dr Seykora began teaching and performing research in the School of Medicine at the University of Pennsylvania, and later went on to become a tenured Professor of Dermatology, a position he still holds today. From being a National Merit Scholar at the University of Chicago in 1982 to receiving the Dean's Award for Excellence in Basic Science Teaching from the Perelman School of Medicine in 2019, Dr Seykora has received an abundance of awards and honours throughout his career.

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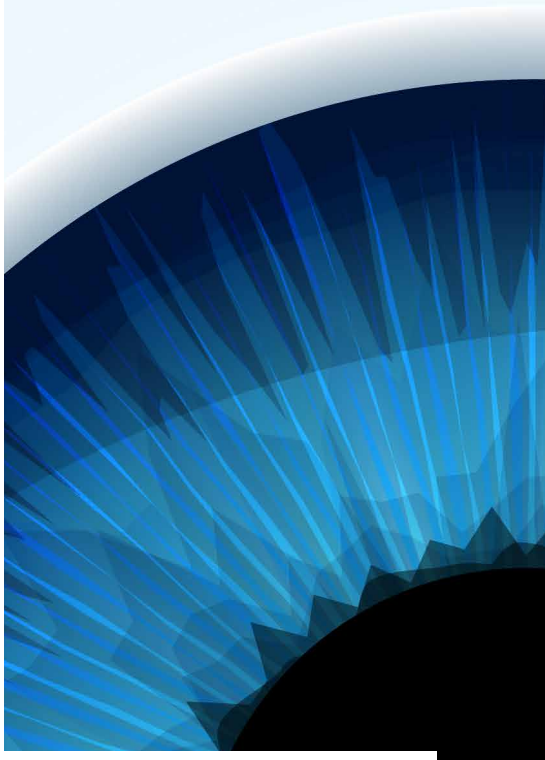
REPAIRING THE CORNEA: A NEW VIEW ON NOVEL THERAPIES



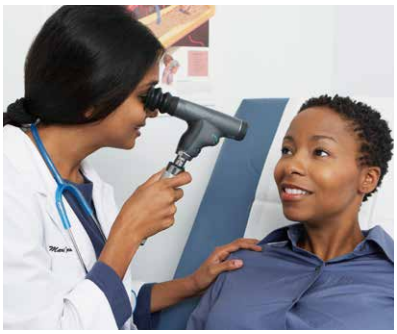
Damage and disease of the cornea are some of the leading causes of sight loss. This can often be remedied is through a transplant with a healthy cornea from a donor. However, donors are few and far between, so innovative solutions are required. **Dr Zi-Bing Jin** from the Beijing Institute of Ophthalmology in China is working on this with some exciting results. Utilising specific small molecules to control cell differentiation, he has discovered a new method for creating the necessary cells for corneal transplant, without the need of a cornea donor.

The Cornea: A Window to the World

Our eyes are complex organs that help us to decipher the world. Vision is created when light bounces off objects and enters our eye through its protective window, called the cornea. The main purpose of the cornea is to focus the light entering the eye by refracting (bending) it onto the retina at the very back of the eyeball. Pupils control how much light enters by expanding or contracting tiny muscles that are constantly working. Images reach the retina upside down because of the curved nature of the eyeball, but this is processed by the brain later on. On the surface of the retina, millions of photoreceptor cells detect the qualities of the light entering. Cone cells allow us to see in colour and detail and are responsible for daylight vision, but rod cells are not sensitive to colour so are only utilised for night vision. All of this information is sent through the optic nerve to the brain, where what we see is compiled and given meaning.

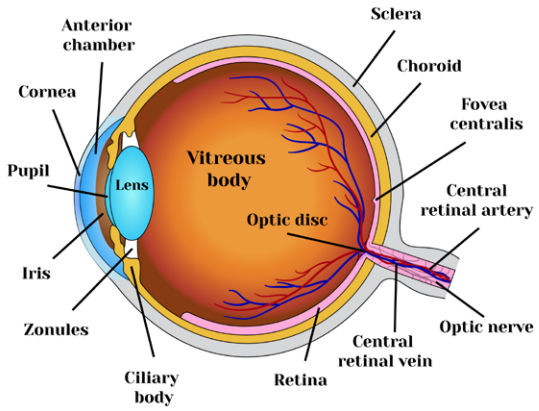
Unusually for the human body, the tissue of the cornea does not contain any blood vessels for nutrient supply but is entirely made up of proteins and cells. This is because it requires complete transparency for clear vision, so nutrients must be distributed in a different way. Oxygen is supplied to the cornea dissolved in tears and then it diffuses throughout the structure to maintain its health. In a similar manner, nutrients are also transported by tear fluid and diffuse from the outside of the cornea, through a special fluid called the aqueous humour, and eventually reach the inside.

The cornea is comprised of five different layers, the outermost being a layer of cells called the epithelium that protects the eye and absorbs nutrients and oxygen. The innermost layer is called the corneal endothelium and unlike the epithelium that can heal itself relatively quickly, the endothelium cannot regenerate. Instead of producing new cells to replace dead ones, endothelial cells stretch to fill the gaps. However, this makes the layer much less dense and less able to regulate the fluid that is present in the cornea. Therefore, damage to the cornea can create excess fluid build-up, which makes it less transparent and causes eyesight to be impaired.

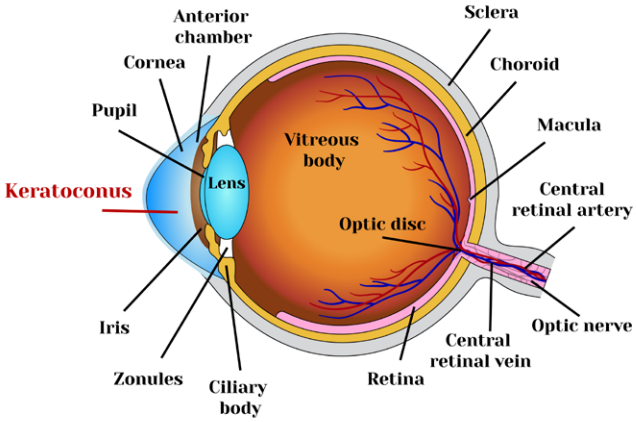


Unfortunately, this is common in old age and in certain diseases. In fact, corneal disorders are the number one cause of blindness globally and they can come in many forms. For example, corneal abrasion is the loss of the epithelium due to trauma and keratitis is caused by the inflammation of the cornea. Keratoconus is a degenerative disease uncommonly prevalent in young adults that causes the cornea to thin and become more coned in shape. These each come with their own complications and difficulties, but dedicated research is targeted at combatting them in their own way.

Normal cornea



Keratoconus



Reprogramming Cells for Corneal Transplantation

Thanks to innovative modern medicine, there are some solutions for those suffering from corneal disorders. Replacing a damaged cornea with a healthy one from a deceased donor through corneal transplantation is the most common therapy. It is thought around half of these procedures are to replace the endothelium of the cornea. Nevertheless, a huge shortage of donated corneas means that fewer than 1.5% of patients who require a corneal transplant are able to receive them from a donor.

However, progress is being made in this field and corneal endothelial cells have been grown from healthy donor tissues and successfully transplanted into models. But this method is still in its early stages and it is difficult to grow a sufficient number of cells for the procedure. Through a method called direct lineage reprogramming, Dr Zi-Bing Jin from the Beijing Institute of Ophthalmology in China is helping to push forward new and exciting techniques for corneal regeneration.
The lineage of a cell is like its family history – what types of cells it differentiated (split and specialised) from to get to its current form and function. In the case of corneal endothelial cells, they originate from neural crest cells, which are able to differentiate into lots of different types of cells in the eye as well as important cells of the nervous system, like glia and neurons.

During the process of lineage reprogramming, the normal route of cell differentiation is altered so that desired and useful cells can be generated from a cell that would not ordinarily produce them. For corneal endothelial cells, this means that elusive donor neural crest cells aren't necessary and other terminally differentiated cells can be utilised. Terminally differentiated refers to cells that have left the cycle of replication and are carrying out their specialised function. If successful, lineage reprogramming could mean that more cell replacement therapies are possible for people with damaged and diseased corneas.

Transcription Factors and Small Molecules

Dr Jin's work focuses on targeting transcription factors. These are proteins that help to control which genes are turned on and off and therefore, can determine what type of cell is produced. Lineage-specific transcription factors have successfully been used to convert terminally differentiated cells into another cell type in test-tube experiments (*in vitro*). In tests on tiny organisms (*in vivo*), an abundance of lineage transcription factors has also been shown to create these conversions.

One way that these transcription factors can be controlled is by using small molecules, which are exactly what they sound like! They are molecules around 1 nanometer in size which aid the regulation of many biological processes; many drugs are classified as small molecules. Recently, they have been used to influence transcription factors to convert connective tissue cells called fibroblasts into other functional cells.

In a novel study, Dr Jin and his colleagues have further developed this method to define specific small molecules that may be applied to therapeutics in the future.

Exciting Results from Diseased Corneal Experiments

Initially, the team screened a multitude of small molecules and eventually found a cocktail that could generate chemically induced neural crest cells from mice fibroblasts, through lineage reprogramming. They found that these chemically induced cells had many of the same features as naturally produced neural crest cells, including which genes they used for protein production (gene expression), their capacity for self-renewal and their differentiation potential. Importantly, they retained the ability to differentiate into corneal endothelial cells. To encourage this second differentiation, Dr Jin had to uncover another mixture of small molecules for the task, which they were successful in finding.

The subsequent chemically induced corneal endothelial cells were also remarkably similar to naturally differentiated ones. Their gene expression, characteristics and function were all alike to primary corneal endothelial cells. This two-step lineage reprogramming strategy is especially exciting as Dr Jin and the team went on to demonstrate it working in a real-world situation.

After reprogramming cells and growing the tissue of chemically induced corneal endothelial cells, they transplanted it into an animal model. They used rabbits that had corneal endothelial disease, which had resulted in bullous keratopathy. Whereas a healthy endothelium layer prevents excessive fluid absorption in the cornea by pumping out liquid, a damaged or diseased one carries out this role ineffectively. As a result, fluid-filled blisters, called bullae, build up on the surface (epithelium) of the cornea and cause the swelling and often pain associated with bullous keratopathy.

Interestingly, the diseased rabbits who were given transplants from these chemically induced cells experienced an almost complete reversal of their corneal opacity. They eventually exhibited clear tissue on their cornea, meaning their sight was restored and the transplant was successful.

Implications for Real-world Application

The results produced by Dr Jin are an exciting step towards improved therapies for damaged corneas and sight loss. He believes that his work could provide a real alternative to relying on donor corneal tissues for transplantation. Fibroblasts could be a new cell source for regeneration and engineering of corneal endothelia and maybe even other tissues that originate from neural crest cells. The small molecules that Dr Jin and his team have identified as integral to this strategy will be incredibly useful for future research on this subject.

The research of Dr Jin and his colleagues at the Beijing Institute of Ophthalmology could go on to make a significant difference in the lives of real people as it is developed further to integrate into therapeutics. By introducing methods to repair corneas, countless people could have their eyesight and quality of life restored.

Meet the researcher

Dr Zi-Bing Jin
Beijing Institute of Ophthalmology
Beijing
China

Dr Zi-Bing Jin is the director of Beijing Institute of Ophthalmology, Full Professor of Capital Medical University (CMU) and Chief Physician at Beijing Tongren Hospital, CMU. Dr Jin focuses on stem cell translational medicine in retinal health and the genetic mechanisms of ocular diseases. His team is dedicated to elucidating the disease mechanisms of inherited retinal degeneration and childhood ocular disorders, translating laboratory technology to improve bedside outcomes, and solving key problems around retinal degeneration. He also focuses on cell reprogramming and transdifferentiation, and this work has led to an exciting new understanding of the mechanisms behind corneal regeneration.

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BUILDING PRECLINICAL MODELS OF RETINAL DEGENERATION IN NON-HUMAN PRIMATES

Mutations affecting the expression of cone photoreceptors can lead to retinal degeneration, which in many cases can result in a permanent loss of vision. However, preclinical models for human retinal degenerative diseases are lacking. **Dr Zi-Bing Jin** and his colleagues study rhesus macaque models of achromatopsia (a congenital disorder characterised by an inability to distinguish colours) and oculocutaneous albinism (characterised by a disorder of melanin synthesis, leading to loss of visual acuity). The animal models utilised in the Jin laboratory offer important opportunities for studies on disease mechanisms as well as therapeutic development.

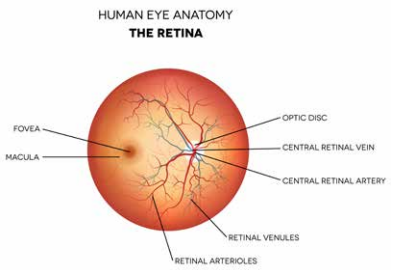
The Macula and the Fovea: High Definition at the Heart of the Eye

Most people are able to see clear, full colour and high-resolution images thanks to the macula, which is located at the centre of the retina. What makes the macula so efficient at detecting light of different wavelengths is the high density of cone photoreceptors expressed in it. Within the central region of the macula lies a 'pit' known as the fovea, which is characterised by a higher density of cone photoreceptors and the presence of approximately 25% retinal ganglion cells. A healthy developed fovea plays a critical role in accurate visual acuity.

Mutations affecting the expression of cone photoreceptors can lead to macular degeneration, which in many cases can result in a permanent loss of vision. Achromatopsia is a congenital disorder characterised by an inability to distinguish colours, low visual acuity, excessive sensitivity to light and uncontrolled eye movements. Several gene mutations have been discovered in patients with achromatopsia. The development of the fovea can be seriously hindered by oculocutaneous albinism (OCA), which is characterised by a disorder of melanin synthesis.

Non-human primates have a macula and fovea closely related to those of humans, so scientists can introduce genetic mutations in these mammals to see whether the deletion of specific genes can lead to the loss of cone photoreceptor function. Dr Zi-Bing Jin and his colleagues focus on stem cell translational medicine in retinal health and the genetic mechanisms of eye diseases, and in particular, those affecting the macular and foveal development.

Dr Jin and his team have created a non-human primate model of achromatopsia by partially knocking out a retinal gene known as *CNGB3*. The gene in question, expressed in the



macular cone receptors, is mutated in patients with achromatopsia. The team at the Jin laboratory hopes that the partial knockout of *CNGB3* in their animal model could replicate the conditions observed in human patients with achromatopsia. Such a model could then be used in drug development studies and to confirm the involvement of the gene in the development of macular degeneration. The team has also developed rhesus macaque models of OCA to study foveal development and to enable preclinical trials of new therapies for OCA.

A Gene Editing Tool to Investigate the Causes of Achromatopsia

Dr Jin and his collaborators published a paper in 2020 describing the design, development and characterisation of their animal model. They used the CRISPR-Cas9 system, a revolutionary tool for generating mutations, for the macular localised knockout (inactivation) of the *CNGB3* gene. The somatic knockout model was initiated by injecting four macaques in the retinal tissue with an adenovirus and the CRISPR-Cas9 system. Following the sub-retinal injection, the team monitored the recovery of the retina at the puncture sites for 30 days. To determine transcriptional changes of *CNGB3* in cone photoreceptors, single cells were isolated from the dissected retina for single-cell RNA sequencing (scRNA-seq) studies. A total of 24 cones were picked from the retina and divided into 13 groups, each group containing at least one and a maximum of three cones.

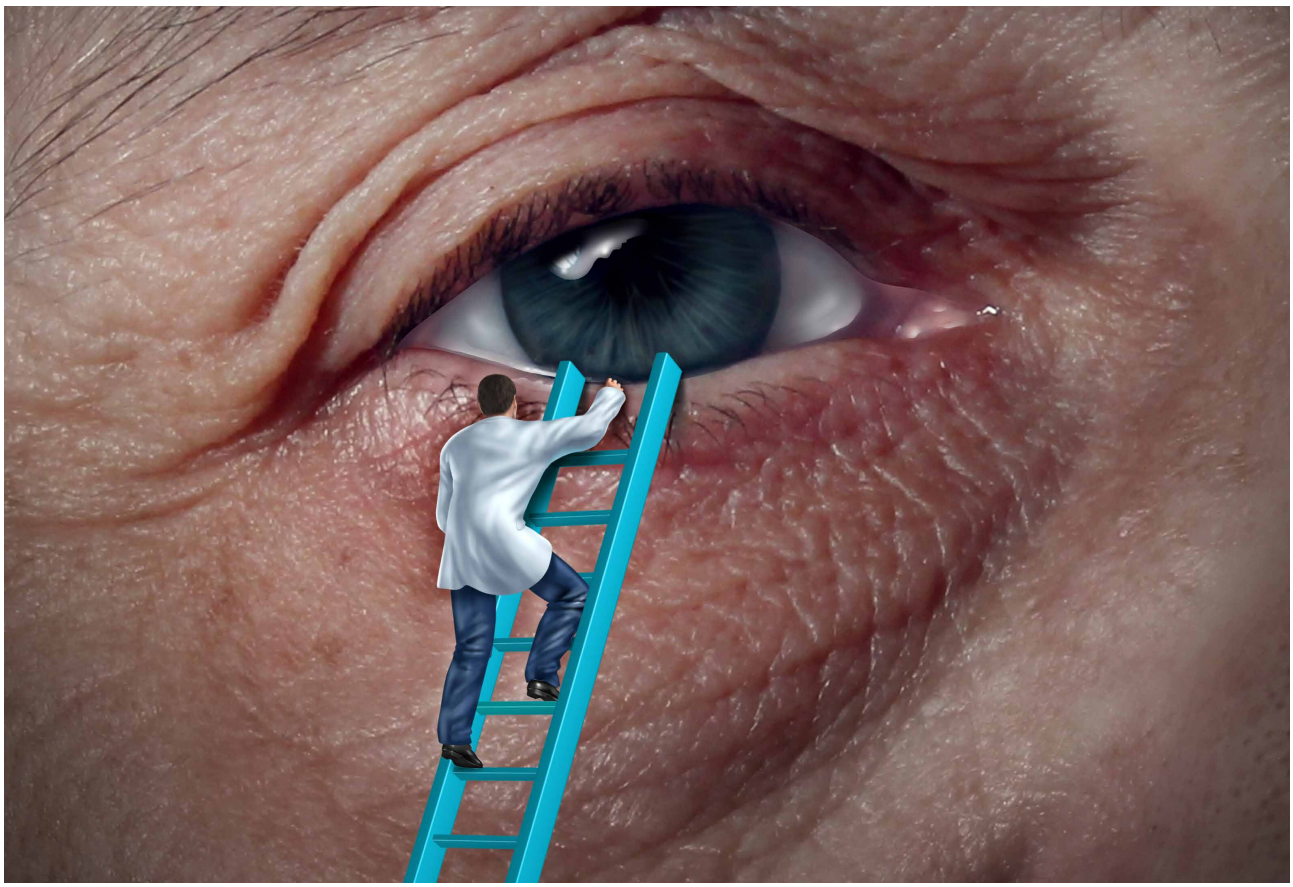
The researchers found that a proportion of treated cells had decreased expressed levels of *CNGB3*, with a targeting efficiency in infected cells of 12.2%, suggesting that subretinal delivery of adenovirus-mediated CRISPR-Cas9 successfully generated a partial knockout of the gene. After measuring the electrical activity in the retina, the team found that, following the *CNGB3* knockout, there was a marked reduction in the expected response from the central retina, suggesting cone dysfunction of the central macula of the primates, consistent with achromatopsia in human patients.

In addition to providing the first non-human primate model for the study of achromatopsia, the paper provided important evidence confirming the safety of CRISPR-Cas9-based gene-editing therapy. The adenoviral-vector-infected areas were only restricted to the retina, leaving other tissues unaltered. This is vitally important in encouraging scientists to perform precise gene editing in vivo. Dr Jin and his collaborators suggested that by improving the targeting efficiency, the CRISPR-Cas9 system could, in future, be used as a 'gene-editing scalpel' in the treatment of macular degeneration and other diseases.

Gene Mutations Impairing Melanin Synthesis

The retinal fovea is a region with a very high density of cone photoreceptors, which is responsible for optimal visual acuity in humans. The human fovea contains approximately 25% retinal ganglion cells, and the synaptic connections between cones, bipolar cells, and ganglion cells in a 1:1:1 ratio, resulting in a high level of precision in the transmission of the visual signals.

In primates, melanin synthesis plays an important role in regulating the proliferation and differentiation of retinal cells. Patients presenting with mutations in a gene known as *TYR* result



in the complete loss or partially reduced activity of the amino acid tyrosine, whose chemical modification is the first step in the production of melanin. Mutations in the *OCA2* gene also cause a partial impairment in melanin production.

The mechanisms of OCA disease have been reported in many mammal species. However, only non-human primates have a foveal structure similar to that of the human retina. The eye structure of rhesus macaques is very similar to that of the human eye, especially because of the presence of a macula and fovea.

In another paper, published in 2020, Dr Jin and his collaborators reported the development of a rhesus macaque model with spontaneous oculocutaneous albinism, with clinical manifestations similar to those of human OCA patients. Albino macaques presenting with low levels of retinal pigmentation had a measured foveal depth that was significantly shallower than that of healthy subjects. Thicker inner retinal layers at the fovea were also found in the albino subjects.

These observations in rhesus macaques are consistent with oculocutaneous albinism in human patients. Whole-genome sequencing from six macaques showed that all the subjects analysed carried mutations in the *OCA2* gene. Additionally, three albino subjects carried another mutation in the *TYR* gene. The researchers confirmed, via *in vitro* assays, that both mutations affected the production of melanin.

Driving Further Developments

The model of OCA developed in the Jin laboratory offers new opportunities for studies on disease mechanisms as well as therapeutic development. Dr Jin and his collaborators pointed out in their paper that, although hundreds of mutations in *TYR* and *OCA2* have been reported in OCA patients, only a few studies have included biological assays to validate the effect of the mutations on melanin synthesis. Studies in humans affected by OCA have shown that photoreceptor layers in the fovea continue to grow, albeit at a reduced rate. These reports suggest that earlier-stage treatment might result in a better outcome for these patients. Dr Jin and his team propose that this hypothesis could now be tested in albino rhesus macaques.

In other developments, the Jin laboratory aims to elucidate the disease mechanisms of children with ocular disorders, translating laboratory technology to improve bedside outcomes. Among other ambitious projects under development, Dr Jin and his team are exploring new, groundbreaking ways of growing key ocular tissues from fibroblasts through small molecules and culturing retinal organoids *in vitro* for the disease modelling of retinitis pigmentosa and retinoblastoma. Following their already highly promising results with the CRISPR-Cas9 system, the team will continue to investigate gene editing for the treatment of complex ocular diseases affecting children and early-onset blindness.

Meet the researcher

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Dr Zi-Bing Jin obtained his MD in 2000 from Wenzhou Medical College. He has also a PhD in Ophthalmology obtained in 2007 from University of Miyazaki. Dr Jin is a Full Professor of Ophthalmology at the Capital Medical University (CMU) and the Director of the Beijing Institute of Ophthalmology. He is also the Chief physician at Beijing Tongren Hospital, CMU, Beijing. Dr Jin aims to elucidate the disease mechanisms of childhood ocular disorders, translating laboratory technology to improve bedside outcomes. Dr Jin and his team research and validate new, groundbreaking ways of growing key ocular tissues from fibroblasts through small molecules and culturing retinal organoids *in vitro* for the disease modelling of retinitis pigmentosa and retinoblastoma. Dr Jin is an active contributor to the wider scientific community, acting as an editor and reviewer for several academic journals.

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THE TRANSLATIONAL ASIAN AGE-RELATED MACULAR DEGENERATION PROGRAM: IMPROVING AGE-RELATED MACULAR DEGENERATION OUTCOMES

Age-related macular degeneration (AMD) is an increasingly common disease that causes significant visual impairment. The implications include socioeconomic burdens for individuals and the population as a whole. Working to elucidate the issues surrounding AMD is **Professor Gemmy Cheung**, who holds senior roles at the Singapore National Eye Centre and the Singapore Eye Research Institute. She has brought together a group of expert scientists to form the Translational Asian Age-Related Macular Degeneration Program. The team is elucidating the mechanisms behind AMD to develop novel therapies, cultivate diagnostics and develop tools to better understand the impact of the disease from patients' perspective.

Age-related Macular Degeneration

Age-related macular degeneration (AMD) is a major cause of vision impairment among elderly individuals worldwide, and is increasingly recognised as a major disease in Asia. AMD is characterised by the growth of new blood vessels beneath the retina, with a tendency to leak, causing sudden vision loss.

Significant limitations exist in current therapies because only the neovascular ('wet') form of AMD is amenable to anti-vascular endothelial growth factor (anti-VEGF) treatment which acts by binding VEGF and thus preventing new vessels growing underneath the retina, to prevent further damage to the retina and central vision. While anti-VEGF therapy achieves stabilisation of vision in the vast majority of cases, only 30-40% of cases achieve significant improvement.

Studying Age-related Macular Degeneration in Asia Through Collaboration

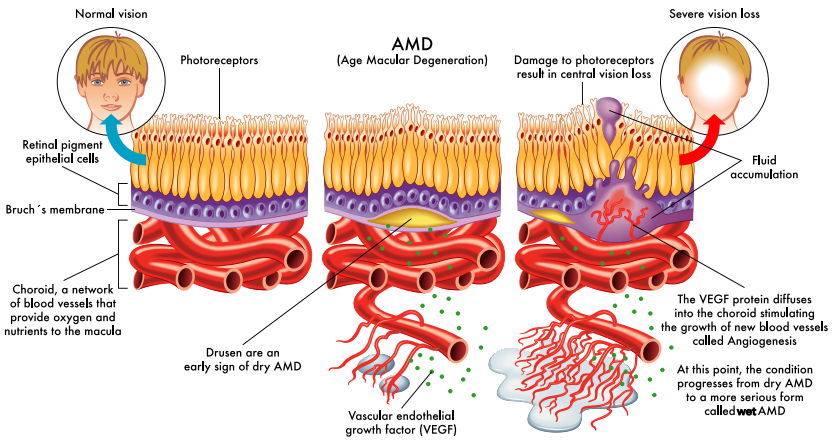
The population in Asia is rapidly ageing, resulting in increasing cases of AMD. One particular subtype of AMD, known as polypoidal choroidal vasculopathy (PCV), makes up about 50% of all AMD cases in Asia, as opposed to less than 20% of cases in white populations. PCV causes similar symptoms and is treated similarly to wet AMD. Currently, there are very limited treatments targeted specifically to this subtype of AMD.

Working to alleviate this burden of disease is Professor Gemmy Cheung Chui Ming and her team of dedicated researchers. They come from a variety of institutions and backgrounds ranging from retinal specialisms to clinical science. Some of their institutions include the Singapore National Eye Centre, the Singapore Eye Research Institute and the Duke-National University of Singapore.

In 2018, the team received an Open Fund Large Collaborative Grant from the Ministry of Health's National Medical

Research Council in Singapore. The program is titled the Translational Asian Age-Related Macular Degeneration Program (TAAP).

According to Professor Cheung, the program aims to 'address the unmet clinical and population needs by a broad-based inter-linked "bench to bedside to population" approach, which will increase our understanding of not only the basic mechanisms of AMD and PCV, but also translate basic research into clinical studies and commercialisation, develop novel diagnostics and imaging, and drive the development of improved therapeutic strategies as well as laying the foundation for future cell-based therapy and rehabilitation technologies.'



The TAAP team has now established a vibrant platform for exchanges between basic scientists, retinal specialists and clinician-scientists. This program has also opened up many training opportunities for clinical fellows, researchers and PhD students. Internationally, TAAP has increasingly gained recognition as one of the most comprehensive translational Asian AMD programs, attracting new academic and industrial collaborations, including:

- Asian Eye Epidemiology Consortium which includes key epidemiology researchers in AMD across 22 countries. They have published a landmark meta-analysis reporting the prevalence of geographic atrophy in the Asian population.

- Asia Pacific Ocular Imaging Society PCV workgroup which includes 15 key clinical researchers in PCV from Asia, Europe and North America with notable scientific contributions to the field of retinal imaging in neovascular AMD and PCV. Two key publications have been published to date, describing i) diagnostic criteria set for PCV without the need for indocyanine green angiography (ICGA), ii) photodynamic treatment planning with OCT. These new publications will allow clinicians and researchers with limited access to ICGA worldwide to evaluate and treat PCV more effectively.

- Fight Retinal Blindness! Registry – Singapore is one of the key Asian sites for this international registry and the members of TAAP are part of the steering committee and scientific writing committee. Through this international database, the team have identified key gaps in current management, such as low presenting vision and poor adherence to treatment, which helps in the planning of strategies to improve treatment outcomes.

Themes Providing Structure to the Objectives

In order to meet its objectives, TAAP approaches research through five unique, but interlinking themes. This wide scope of research is aimed to lead to much a broader and deeper understanding of AMD, biologically, technologically and sociologically.

The *Population Health* theme is headed by Professor Tien Yin Wong and Professor Ching-Yu Cheng. In their well-established population cohort, they will report the 12-year incidence and risk factors of AMD, and evaluate the genetic background associated with AMD.

Associate Professor Xiaomeng Wang leads the theme on *Pathophysiology*, which investigates disease-causing pathways and mechanisms. They have identified several novel molecules which may play important role in abnormal blood vessel growth (angiogenesis) and scarring in AMD and PCV.

Professor Leopold Schmetterer, Professor Caroline Chee and Professor Cheung work on *Novel Imaging techniques and Biomarkers.* Their approach includes developing novel hardware and algorithms for analysis, as well as identifying imaging features that can prognosticate and guide treatments.

In the *Therapeutics* theme, led by Professor Cheung, Associate Professor Colin Tan and Dr Kelvin Teo, clinical trials will be conducted to evaluate cost-effective and personalised treatments and their long-term outcomes.

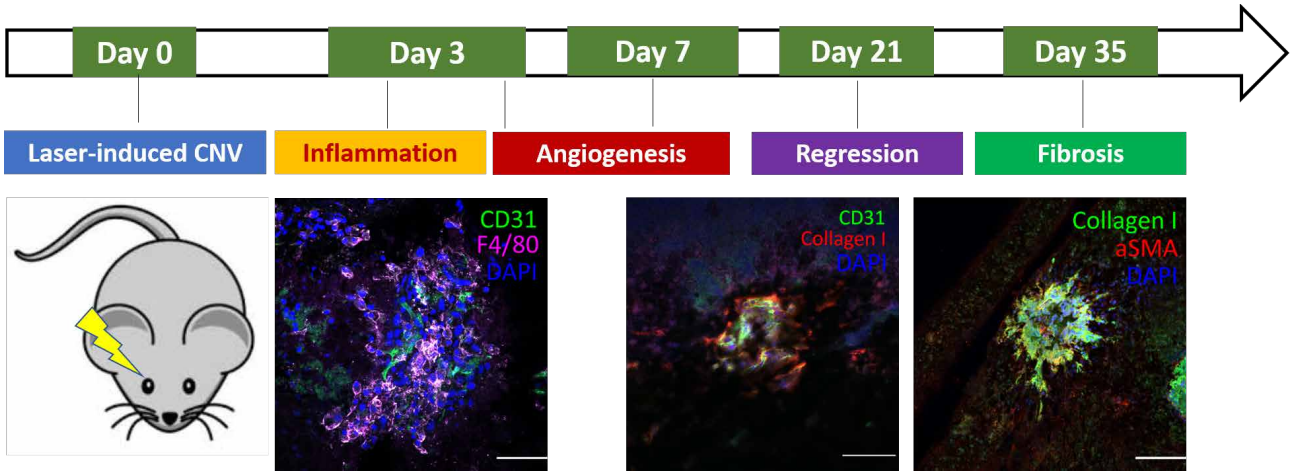
In the theme evaluating *Quality of Life*, led by Professor Ecosse Lamoureux and Professor Dan Milea, a cloud-based, computerised system will be developed to assess the impact of AMD on patients.

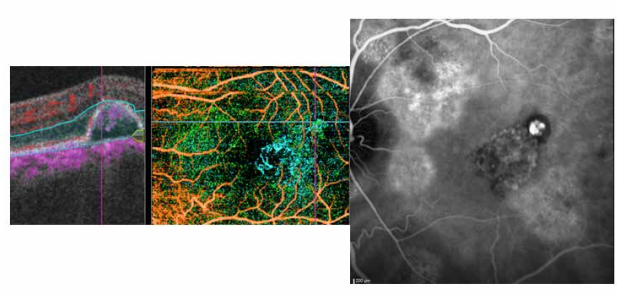
Novel Findings from the Translational Asian Age-related Macular Degeneration Program

Just three years into their project, the TAAP team have uncovered some interesting results:

Understanding Disease Burden and Risk Factors. The prevalence and risk factors for subphenotypes of AMD in Asians is unclear. The TAAP team led a large meta-analysis which reported the prevalence of macular atrophy in Asian individuals. Similarly, the prevalence of the PCV subtype has been poorly evaluated in population studies due to the reliance on invasive angiography. The clinical imaging team identified and validated a non-invasive set of diagnostic criteria that will allow the estimation of PCV cases in population studies. Concurrently, the imaging team has reported that optical coherence tomography angiography (OCTA) can non-invasively detect non-exudative neovascularisation, which is an important precursor lesion.

Developing Therapy. Several key targeted pathways of interest have been identified through genetics work and pilot metabolomics studies from





patients' serum or ocular fluid. A key target is leucine-rich alpha 2 glycoprotein 1(LRG1) which plays a role in angiogenesis and fibrosis. Neutralising antibodies to LRG1 have been developed and will be evaluated in animal models. Several clinical trials are being conducted concurrently to identify the efficacy of various anti-VEGF regimens individually and in combination with photodynamic therapy.

Recently, the team completed a randomised control trial over one year. They compared the outcome of PCV patients on a fixed regimen of the anti-VEGF drug, aflibercept, with those on a personalised and flexible treatment plan. Patients who received personalised therapy had, on average, better outcomes. These patients experienced a greater reduction of central subfield thickness and more had closure of polypoidal lesions, both of which are indicators of improved macular health. Importantly, many achieved improved best-corrected visual acuity, meaning their visual impairment was reduced.

Diagnostics and Imaging Biomarkers. The TAAP team led an international panel and developed a new and simpler way to diagnose PCV using a technique called optical coherence tomography (OCT) that uses low-coherence light to create high resolution, two- or three-dimensional images of the inner eye. Often AMD is diagnosed through ICGA, which requires an injection of indocyanine green dye that fluoresces under certain wavelengths to create images of the eye. The new OCT technique removes the need for ICGA and is deemed to be a simpler method. Aiding this diagnostic method are additional imaging biomarkers that the team developed. These can be used to predict how badly the vision of a patient with AMD/PCV will be affected by looking at images of their eye.

The team have built prototypes with polarisation-sensitive OCT and will evaluate the performance of this device in detecting ß-amyloid deposition in the retina. Another prototype combining megahertz OCT with OCTA technology will be used to evaluate choroidal vasculatures. In clinical patients, the team specialises in evaluating choroidal parameters and combines novel algorithms such as automated choroidal vascularity index and choroidal volume with traditional imaging parameters, as choroidal features may have additional prognostic value in PCV.

Developing Cost-efficient Care Pathways. The advent of anti-VEGF therapy has revolutionised the treatment for AMD in preventing blindness. Much is still unknown, especially about the long-term retreatment requirements. Until now, there have been limited data available from real-world studies in Asia. The team has published real-world data from the Singapore National Eye Center audit as well as Fight Retinal Blindness Registry, and highlighted patterns that may explain suboptimal treatment.

Continuing the Work

Professor Cheung and her team at TAAP are continuing their research and pushing the boundaries in their field. By clarifying mechanisms of AMD disease, progressing diagnostics and treatments and better understanding how this widespread ailment impacts those living with it, TAAP has and will continue to elucidate how patient outcomes can improve. Their work is sure to make a real difference in the lives of visually impaired people in Singapore and around the world.

The vision of this ambitious program is to develop paradigm shifts in the conceptualisation and understanding of Asian AMD, including PCV, that will lead to novel classification and evidence-based cost-effective diagnosis, prevention and treatment strategies in 5 years. Their long-term goal is to reduce AMD-related blindness by 20% in 10 years.

Meet the researchers

TAAP LEAD PRINCIPAL INVESTIGATOR
Professor Gemmy Cheung Chui Ming
Senior Consultant and Head, Medical Retina Department, Singapore National Eye Centre
Professor, Duke-NUS Medical School, National University of Singapore
Head, Retina research group, Singapore Eye Research Institute

TAAP THEME PRINCIPAL INVESTIGATORS (from left to right)

Professor Tien Yin Wong, Professor Ching-Yu Cheng, Associate Professor Xiaomeng Wang, Professor Leopold Schmetterer, Professor Caroline Chee, Dr Kelvin Teo, Associate Professor Colin Tan, Professor Ecosse Lamoureux, Professor Dan Milea

Over her impressive career, Professor Gemmy Cheung Chui Ming has achieved MBBS, MRCOphth, FRCOphth and MCI qualifications. Currently, she is a Professor at Duke-National University of Singapore Medical School, National University of Singapore, Head and Senior Consultant of the Medical Retina Department at Singapore National Eye Centre and Head of the Retina Research Group at the Singapore Eye Research Institute. Professor Cheung has held numerous positions and received multiple prestigious awards for her research in ophthalmology. Her dedicated studies into retinal health, specifically AMD, have clarified causes and opened novel diagnostic and therapeutic options, and have led to multiple presentations, publications and collaborations at an international level. She has published more than 250 peer-reviewed articles and more than 10 book chapters. She has also given more than 100 invited lectures and served as an instructor on courses at the Asia-Pacific Academy of Ophthalmology, American Academy of Ophthalmology Congress.

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FUNDING

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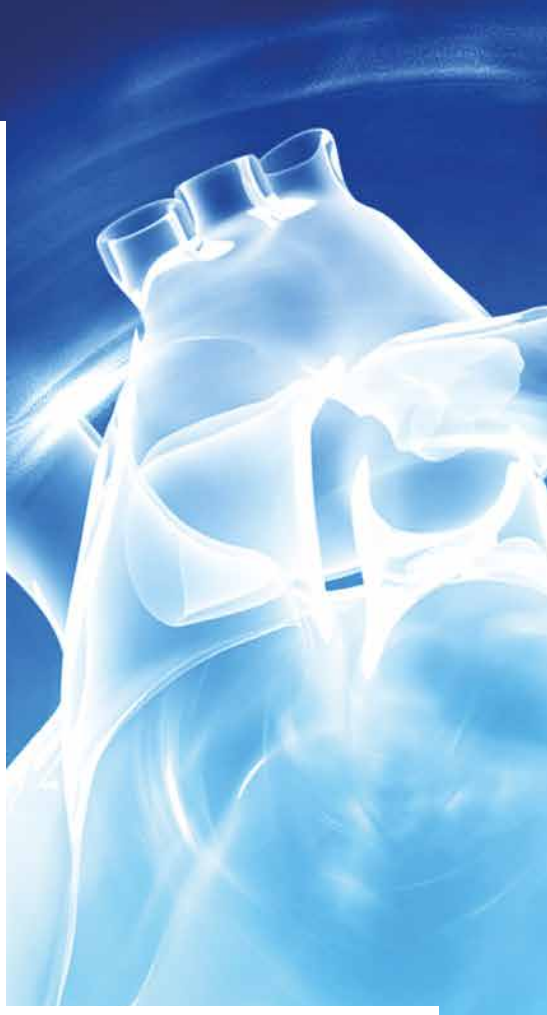
A HYDROGEL WITH THE ABILITY TO RECOVER HEART FUNCTION



The human heart is a muscle, and like all types of muscles, it can be injured. In humans, heart muscle is not able to regenerate after injury, and this can lead to heart disease which develops over time, eventually leading to an untimely death. A team of researchers, **Doctor Claudine Bruck** (Prolifagen), **Professor Edward Morrisey** (Department of Medicine and Cell and Developmental Biology) and **Professor Jason Burdick** (Department of Bioengineering) at the University of Pennsylvania, have collaborated to develop a novel therapy to regenerate damaged heart muscle.

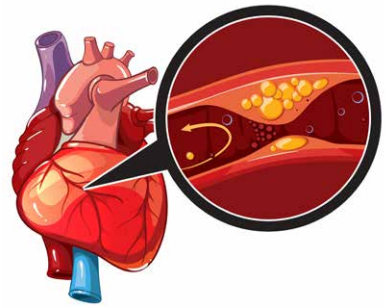
The Challenges of Treating Heart Disease

Heart disease is a major cause of mortality around the world and the World Health Organisation estimates that it results in close to 18 million deaths annually. Heart attack, the common terminology for myocardial infarction (MI), contributes to many cases of heart disease. Although advances in management and surgical intervention have improved survival rates, chronic heart failure (HF) is a serious consequence of MI and at least half of sufferers do not survive past five years. Repair of an MI is primarily through formation of scar tissue, as heart muscle cannot regenerate after injury. HF often develops progressively after an MI, which can eventually lead to premature death.

Cardiomyocytes are the key cells of the heart muscle. They terminally differentiate after foetal development and stop proliferating around the time of birth. Upon terminal differentiation, cardiomyocytes are unable to proliferate due to their exit from the cell cycle. Thus, when the adult heart is injured, cardiomyocytes die and cannot be replaced through proliferation or regrowth from existing cells.

Traditionally, only heart transplantation has been a cure for HF but this is costly and challenging, and therefore, not an option for all patients. The regeneration of cardiomyocytes following MI would be one way of treating damaged hearts and HF. This has long been needed and much research has been focused over the years on stem cell therapy to produce new cardiomyocytes to repair damaged heart muscle. However, the development of an effective stem cell therapy has not yet been achieved due to numerous obstacles.

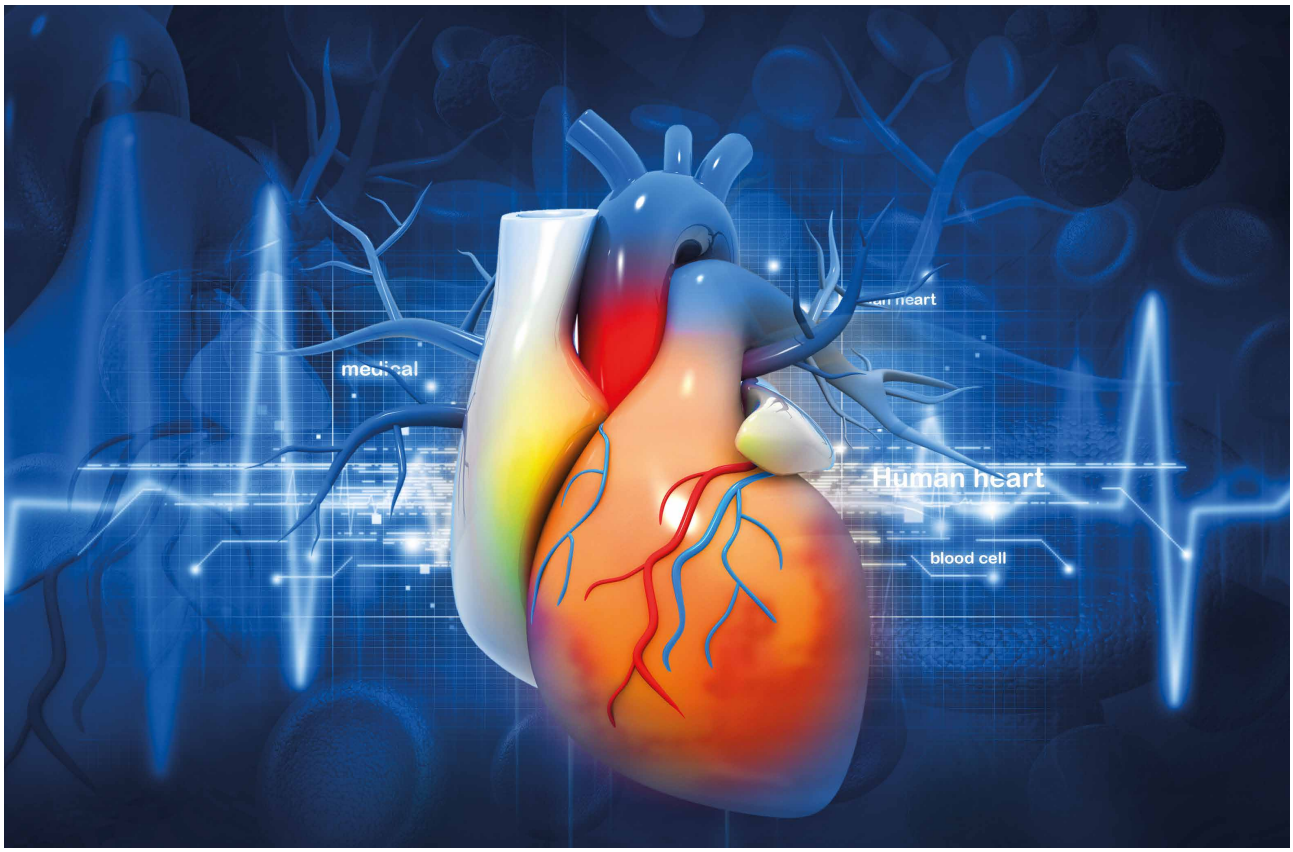
Professor Edward Morrisey of the Department of Medicine and Cell and Developmental Biology at the University of Pennsylvania has invented a new cardiac regeneration technology along with Professor Jason Burdick of the Department of Bioengineering at the same institution. Professor Morrisey has co-founded the biotechnology company Prolifagen, with the drug development



expertise of the company's CEO, Doctor Claudine Bruck to further develop this exciting technology for use in the treatment of HF in humans.

The Science Behind an Emerging Cardiac Therapy

In a 2016 research paper, Professor Morrisey and colleagues described the proliferation of cardiomyocytes in mice. MicroRNAs (miRNAs) are a class of small, non-coding RNAs



that function in regulating gene expression by binding to target mRNA (messenger RNAs), destabilising them and initiating translational silencing. Professor Morrisey's group discovered a miRNA cluster called miR302-367 that is expressed in early mouse cardiac development and plays an important role in the proliferation of cardiomyocytes during embryonic development.

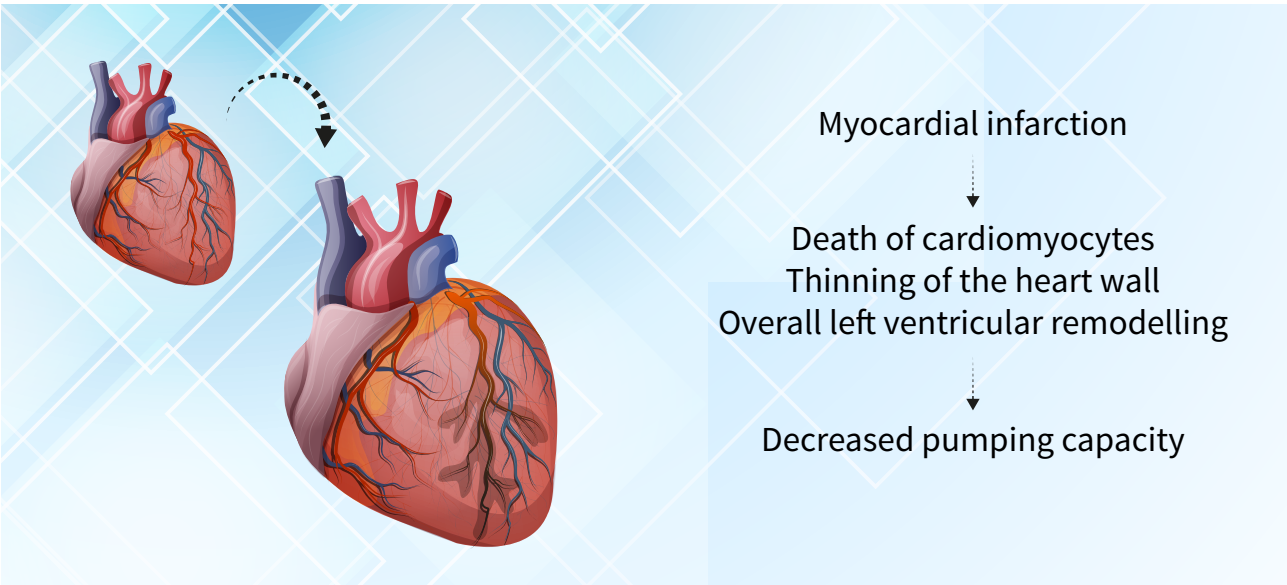
The expression of miR302-367 decreases during heart development, which corresponds to decreasing cardiomyocyte proliferation. Importantly, over-expression of miR302-367 led to increased cardiomyocyte proliferation in mice as compared to controls. The researchers demonstrated that miR302-367 works by targeting components of the highly conserved Hippo signal transduction pathway. This pathway is known to control organ growth by regulating cell proliferation, apoptosis and stem cell renewal. High-throughput sequencing of mouse mRNA hybridised to miR302-367 Mst1, a core component of the Hippo signalling pathway, as a primary target of miR302-367. The team hypothesised that miR302-367 regulates cardiomyocyte proliferation by inhibiting the Hippo signalling pathway. Moreover, the importance of this pathway in cardiac regeneration has also been reported by others.

Further experiments revealed that re-expression of miR302-367 in the adult mouse heart resulted in the reactivation of the cardiomyocyte cell cycle, an increase in the number of cardiomyocytes and regeneration. When the team induced MI in these adult mice, a reduction in scar formation was observed as compared to controls. However, the team also noted that long-term expression of miR302-367 caused dedifferentiation and dysfunction of cardiomyocytes and, therefore, compromised cardiac function.

Following this, the team demonstrated that transient treatment of mice with miR302-367 mimics (miRNA that silence/repress target genes) administered daily for one week, resulted in increased mouse cardiomyocyte proliferation after MI, decreased fibrosis, and increased physiological function while at the same time not causing cardiomyocyte dedifferentiation. Importantly, there was recovery of cardiac function as measured by end-systolic, end-diastolic volume and ejection fraction. The results of these experiments led the team to conclude that there is potential for miRNA-based therapy to activate cardiomyocyte proliferation and cardiac regeneration following injury.

A Bio-engineered Injectable Therapy to Treat Heart Disease

Following the promising results of the miRNA study, Professor Morrisey and Professor Burdick collaborated to develop a miRNA-based therapy for the treatment of MI. The research was published in a 2017 paper where the team proposed delivering miR302 mimics to the heart via an injectable hyaluronic acid hydrogel. Hydrogels are water-based polymer networks, which can encapsulate drugs that are then released over time after the gel is injected into the tissue. Professor Burdick's team developed a hydrogel



Myocardial Infarction and Heart Failure

with modified hyaluronic acid that exhibits shear-thinning characteristics. This allows the gel to flow during injection, and when it reaches the target site, the gel reassembles almost instantaneously.

The team studied neonatal mouse cardiomyocytes which had been treated with the gel-miR302 complex and found that the complex significantly enhanced proliferation compared to controls that were either hydrogels with no specific sequence (gel-miR-NC) or hydrogels alone. The hydrogel release profile after injection into the heart muscle indicated that the gel-miR302 complex was locally present for approximately one week from a single injected dose, whereas, in the previous study, systemic daily dosing (that is, affecting the whole body) of miR302 mimics through intravenous injection was needed.

To determine whether the cardiomyocyte proliferation following gel-miR302 treatment yielded new cardiomyocytes after MI, the team conducted a set of experiments to trace the lineage of individual cardiomyocytes using fluorescent labelling to track daughter cells from parent cells. MI was induced in mice and then injected with either gel-miR302 or gel- miR-NC (control), adjacent to the infarction site in the border zone. Mice injected with gel-miR302, but not gel- mIR-NC, showed that individual cardiomyocytes replicated into multiple cardiomyocytes in the infarcted border zone at day 28 post-injection. These data indicate that miR302 treated cardiomyocytes proliferated or grew after an MI and treatment.

The team further investigated the effect of the gel-miR302 injection on heart physiology after MI. The team selected adult mice treated with gel-miR302, gel-miR-NC or a no-hydrogel control after MI. Four weeks post-MI, echocardiography and a number of cardiac measurements were performed to assess cardiac function in treated mice. The findings showed that 4 weeks after MI, mice treated with gel-miR302 had decreased cardiac end-diastolic and end-systolic volumes (39% and 50%, respectively), improved ejection fraction (32%) and fractional shortening (64%) as compared with controls. Furthermore, echocardiography showed improved heart wall movement in gel-miR302 treated mice. The team concluded that the gel-miRNA based delivery system can be effective in promoting cardiac regeneration after MI and this method is most important as it provides a targeted and localised delivery as opposed to a systemic delivery which can lead to off-target effects.

Next Steps Towards Use in Humans

This is an exciting time for Prolifagen as they implement the next steps towards fine-tuning this new therapy as a suitable treatment in humans. Currently, the team is conducting pharmacokinetic and pharmacodynamic studies in porcine (pig) models as a proof-of-concept for human trials. For the Phase I porcine experiments, the researchers will use direct needle injections to deliver the gel-miR302 complex, however, for human trials, they propose a catheter administration.

The porcine heart is of a similar size to the human heart and will, therefore, be used to measure the induction of cardiomyocyte proliferation following injection of gel-miR302 at two different concentrations. Preliminary experiments have shown induction of cardiomyocyte proliferation following gel-miRNA302 treatment of porcine hearts. Once the porcine Phase I studies are complete the Prolifagen team aims to test the safety and efficacy of gel-miR302 in the porcine MI model.

Following success in these studies, preclinical toxicity studies will be completed ahead of early phase human trials. This novel therapy for the treatment of heart failure is a promising one with the potential of improving the outcomes for millions of patients that have suffered MI.

Meet the researchers

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Doctor Claudine Bruck received her PhD in Biochemistry and Molecular Virology at the University of Brussels in 1982 and completed her postdoctoral research at Harvard Medical School. Doctor Bruck has 30 years of experience leading the pharmaceutical industry in vaccine research and drug development.
She is the former vice-president and Head of GSK Ophthalmology R&D and currently the CEO and co-founder of Prolifagen where she is managing the development of the company's cardiac regeneration technology.

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Prolifagen
life again

USING QUANTITATIVE TOOLS TO LEARN MORE ABOUT GENES

Applying mathematics to biology can help reveal the elusive details of life. **Professor Wilfred D. Stein** and his colleagues from the Hebrew University of Jerusalem and institutes worldwide have used quantitative tools to tackle topics ranging from cancer treatment to drug-resistant malaria, and lately, the evolution of the human genome. His extensive and broad work has supported scientists in many different areas of biomedicine and will leave a lasting impact on life science.

Biomathematics: A Far-reaching Science

The work of Professor Wilfred D. Stein, Professor Emeritus at the Hebrew University of Jerusalem, has covered ground far and wide, from the genetics of cancer to drug-resistant malaria. A common thread in his work has been the use of mathematics to find objective answers to key questions. 'What I do is to apply quantitative methods to problems of biology,' explains Professor Stein. The mathematics used by Professor Stein tends to focus on tumour growth, protein interactions and gene evolution, aspects of biology which are very amenable to mathematical modelling.

One of Professor Stein's recent projects on gene ageing emphasises the utility of quantitative tools in studying biology. Estimating the age of genes can help further our understanding of their role but would be nigh on impossible without the quantitative tools which have been developed by biomathematicians.

A Look Back in Time

A gene's age is determined by how far back in time it can be first identified in an ancestor of the organism in question. This can be achieved using the extensive and widely available genomic databases. Taking advantage of these resources, in 2018 with his colleague Professor Litman, Professor Stein published a list of the ages of all the protein-coding genes and many of the non-protein coding genes in the human genome.

Professor Stein wanted to use this gene ageing technique to learn more about cancer-associated genes and when they arose in the evolutionary tree. Genes are a tricky thing to study, and only recently have scientists developed ways to do so. Genomic phylostratigraphy is a statistical method which looks at the way genes evolve by comparing them across species and ancestors throughout time. Genes, like us, are the descendants of ancestors, not exact copies, but similar enough to see their relationship.

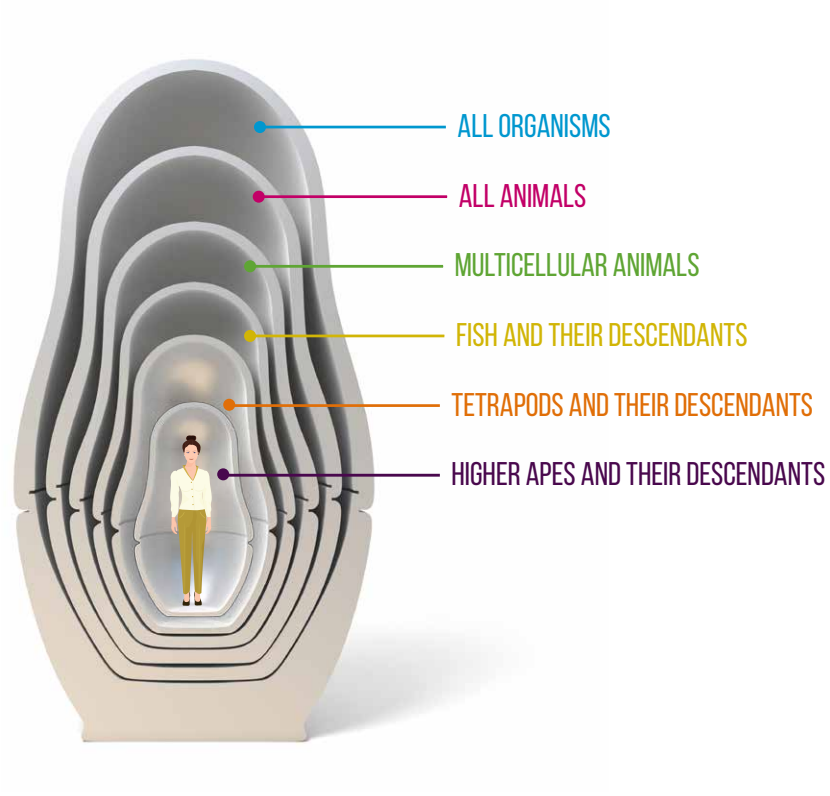
When studying evolution, scientists can draw up a tree of ancestors for an organism, much like a family tree.

Humans, at the very top of the tree, are of the order primates, which evolved from the placental mammals, which evolved from terrestrial vertebrates, and so on, all the way back to the last universal common ancestor. This common ancestor, known as LUCA, was likely a single-celled organism living in the deep ocean which would eventually give rise to every organism we know today.

Organisms on the tree can then be sorted into clades, a group which shares a recent common ancestor. Humans and chimpanzees are in the same clade because we share an ancestor, just like how you and your cousins are in the same family when you share a grandfather or a grandmother.

'Clades are nested, one in another,' Professor Stein notes. New clades form as evolution proceeds, new

‘I have been able to estimate the ages of the genes that have been added, over the ages, to the evolving human genome.’



New clades form as evolution proceeds. The outermost clade contains all living organisms.

characteristics appear and populations diverge and evolve independently. Like sets of nesting dolls, the outermost clade, or the largest doll, contains all living organisms. Each clade contains all the descendants of one common ancestor. In the family tree example, your extended family clade is comprised of you and all the descendants of your grandfather, while your immediate family clade only includes you and your siblings, the descendants of your father or mother.

The organisms which have not ‘evolved out’ of their clade can be considered as being in a phylostratum level. ‘A phylostratum level is the set of organisms that first appeared in a newly-formed clade and remained in that clade, while some of their descendants evolved new characteristics to form the organisms of the next succeeding clade,’ explains Professor Stein. ‘The organisms that remained behind, between one clade and the next, form the new phylostratum level’.

This concept of phylostratification is a valuable tool for scientists who attribute ages to genes. Genes are passed on to descendants in vertical evolution. Over time they may change slightly, perhaps making their function more efficient, but still effectively doing the same job. Some changes, however, can allow the taking on of a new function, making this into a brand new gene. Alternatively, a quite new gene can emerge, an ‘orphan gene’, arising from a DNA sequence that did not code for anything. A new gene, and its descendants that retain the same function, are known as orthologs.

So, a gene can be given an age by identifying the phylostratum where its founder gene, the earliest ortholog, first appeared. These ‘origin’ orthologs are found by searching online databases of entire genomes of living organisms, using a genomics tool called BLAST. By inputting the sequence of your gene of interest, BLAST finds genes with similar sequences which are likely to be the gene’s ancestor. By identifying the earliest phylostratum that this ancestor is in, we can obtain an estimate of how old it is.

Identifying gene ages using orthologs enables us to develop our understanding of evolution and has helped us learn more about the relationships between organisms. However, while this technique is useful, many think it is too much of an inaccurate science, and disputes in the scientific community mean that there is no agreed list of orthologs.

Ancient Cancer Genes

‘By building on a consensus of the findings of the ortholog researchers, one can estimate the ages of the genes that have been added, over the ages, to the evolving human genome,’ elaborates Professor Stein, ‘and I have used this information to estimate ages of genes that, when mutated, drive cancer in the human body.’

The genetics of cancer has been of interest to researchers ever since cancer was first found to have a heritable element. Discovering when these genes may have arisen in evolutionary history may help us understand more about them.

Cancer-associated genes can be grouped into two classes with distinct and important roles in cell survival. Caretaker genes control things like the frequency of mutations which occur when cells replicate, while gatekeeper genes control cell growth. Mutations in these genes can result in a dysregulation of these important processes and cause excessive cell proliferation, or tumour growth. There are some thousand or more cancer-associated genes, many of which contribute just slightly to the possibility that someone will develop cancer.

$$min = (\exp(g \cdot (\ln(r)/ \quad g \cdot (1 + r))) + \exp(-d \cdot (\ln(r) / g \cdot (1 + r))) - 1$$

$$min = (\exp(\ln(r)/ (1 + r)) + \exp(-\mathrm{r} \,.\, (\ln(r)/(1 + r)))-1$$

Equations illustrating the growth of cancers in response to chemotherapy.

With all the available tools at his disposal, Professor Stein and his co-workers began looking for the point at which cancer-associated genes arose in what would eventually become the human genome. They found that not only did cancer genes appear much earlier than would be expected, the genes associated with different types of cancers appeared at different points in time.

Carcinomas (cancers of the inner and outer linings of the body) and lymphomas (that arise from immune cells) are associated with genes regulating cell proliferation which appeared in the genome when organisms became multicellular. Many of the lymphoma specific genes appeared slightly later, at a point when the immune system developed in fish. Indeed, Professor Stein suggests that the earlier appearance of carcinoma genes is due to their more general function in terms of regulating activity at a point when cell differentiation was emerging in the first multicellular organisms.

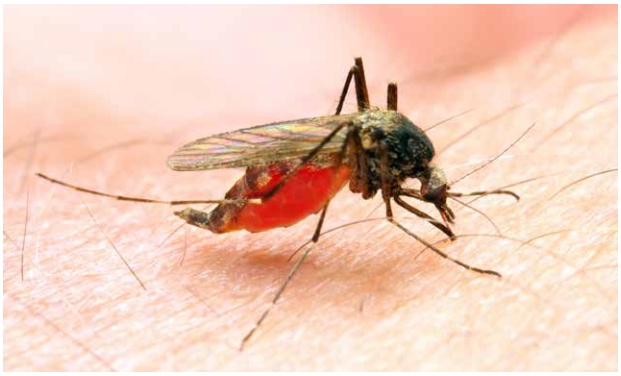
Professor Stein's research adds to our current understanding of the way the human genome evolved from all our previous ancestors. With cancer, it may be the case that mutations cause a breakdown in communication between our originally unicellular genes and multicellular genes. Uncovering these links can help in combatting this disease.

Predictive Thinking

Professor Stein's work didn't start there, however. Before his interest in gene evolution became fully realised, he was applying algorithms to better understand tumour growth. He derived a set of equations to measure the rate at which a tumour regrows following chemotherapy and demonstrated how this rate better predicts the patient's outcome. This is in contrast to current approaches, where doctors look at the extent to which a tumour is reduced by treatment. Professor Stein further elaborates, 'We found that the best predictor of the subsequent fate of patients treated with chemotherapy was the rate of regrowth of the tumour.'

In many cases, if a tumour is not reducing significantly in response to chemotherapy, the treatment is deemed ineffective and may be stopped. Professor Stein suggests that by looking at the regrowth rate, we can continue treatment if the tumour is growing slower than it would using other drugs. This could help prolong the life of patients and may mean that more chemotherapy drugs in the pipeline have a chance at making it to the clinic.

These kinds of mathematical tools can help determine better clinical endpoints, the point at which treatment is considered effective, and can help us make more informed decisions about what to look for when assessing drug efficacy. 'Only if there is a better drug out there, with a lower growth rate than the drug being used should the therapy be changed,' says Professor Stein, 'I think this is one of the most important of my findings over the years.'



Malaria and Beyond

Professor Stein's research has taken him through a myriad of important topics. He has also dedicated time to the study of drug-resistant malaria and developed kinetic equations to show how the parasite's transporter proteins remove antimalarials from their site of action. Knowing this, scientists can now begin researching novel drugs to combat what has become an anti-malarial resistance crisis.

Currently, Professor Stein is applying the gene ages approach to elucidate the evolution of hair cells that were an invention of the mammals. Thus, he very much remains an active contributor to research and continues to bring his unique quantitative perspective to problems of biology.

Meet the researcher

Professor Wilfred D. Stein
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Professor Wilfred D. Stein received his MSc in Physiological Chemistry from the University of Witwatersrand in 1954 and his PhD from King's College London in Biophysics in 1958. His postdoctoral work took him to the Universities of Cambridge and Michigan Ann Arbor before he took up the position of Assistant Professor at the University of Manchester. In 1968 he joined the faculty of the Hebrew University of Jerusalem where he worked until his retirement, teaching biochemistry, biophysics, and physiology. His research has focused on using quantitative tools to investigate problems of biology and has ranged from the kinetics of tumour regrowth to determining the ages of genes with phylogenetic techniques. Professor Stein is the author of more than 300 peer-reviewed publications and nine books.

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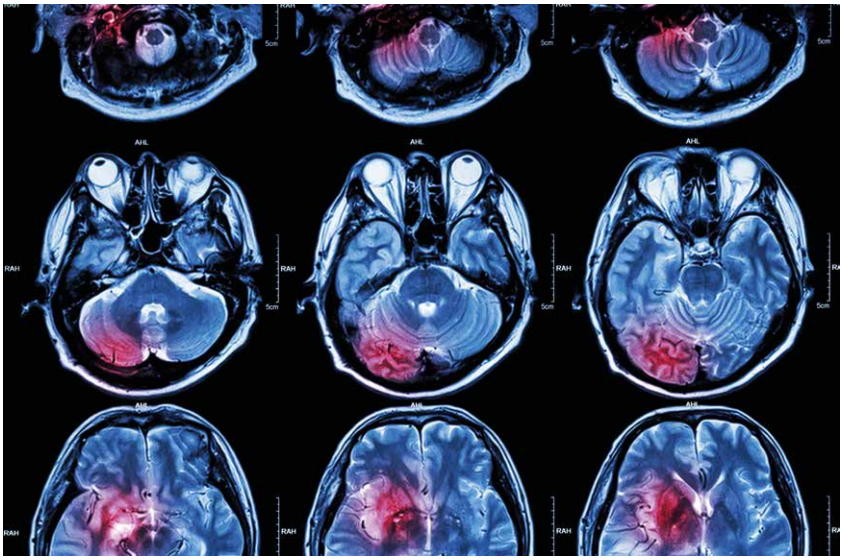
ESTABLISHING METHODS FOR MEDICAL IMAGING AND RESEARCH: COLLABORATIVE RESEARCH CENTRE 1340

As medicine progresses, new techniques are needed to visualise abnormal extracellular structures with greater specificity and resolution. Currently, there is a clear lack of molecular tools to image extracellular structures with the detail needed for early diagnosis of various medical conditions. Based at the Charité – Universitätsmedizin Berlin, the Collaborative Research Centre 1340 represents a large collaboration of researchers from institutions across Berlin, who are working to establish new methods for medical imaging and research at the anatomical and molecular levels.



Developing New Imaging Probes to Better Understand Disease

In medical imaging, techniques such as magnetic resonance imaging use agents that enhance contrast for clearer and more effective images. However, these substances are in most cases non-specific. Disease-specific imaging is needed to improve treatment planning and to improve the assessment of the course of an illness during therapy. However, very few specific agents or probes have been approved for clinical imaging in the last 30 years.

The Matrix in Vision Collaborative Research Centre (CRC) has brought together different researchers from across Berlin. Specialising in various areas of biochemistry, physics and medicine, the researchers focus on the establishment of novel imaging techniques targeting the extracellular matrix (ECM). This is critical due to the role of the ECM in the development and progression of disease, as we will now consider.

The Extracellular Matrix

Most of the recent imaging agents and probes have been components that bind to the cell surface. After the cell surface, the ECM is the next important tissue component that can be targeted by different imaging techniques. The ECM mainly determines the biomechanical properties of tissue and is, therefore, a suitable target for molecular and biophysical imaging approaches.

The ECM functions as a scaffold in which cells of the tissues and organs of the body are embedded. In addition to providing structure, the ECM facilitates

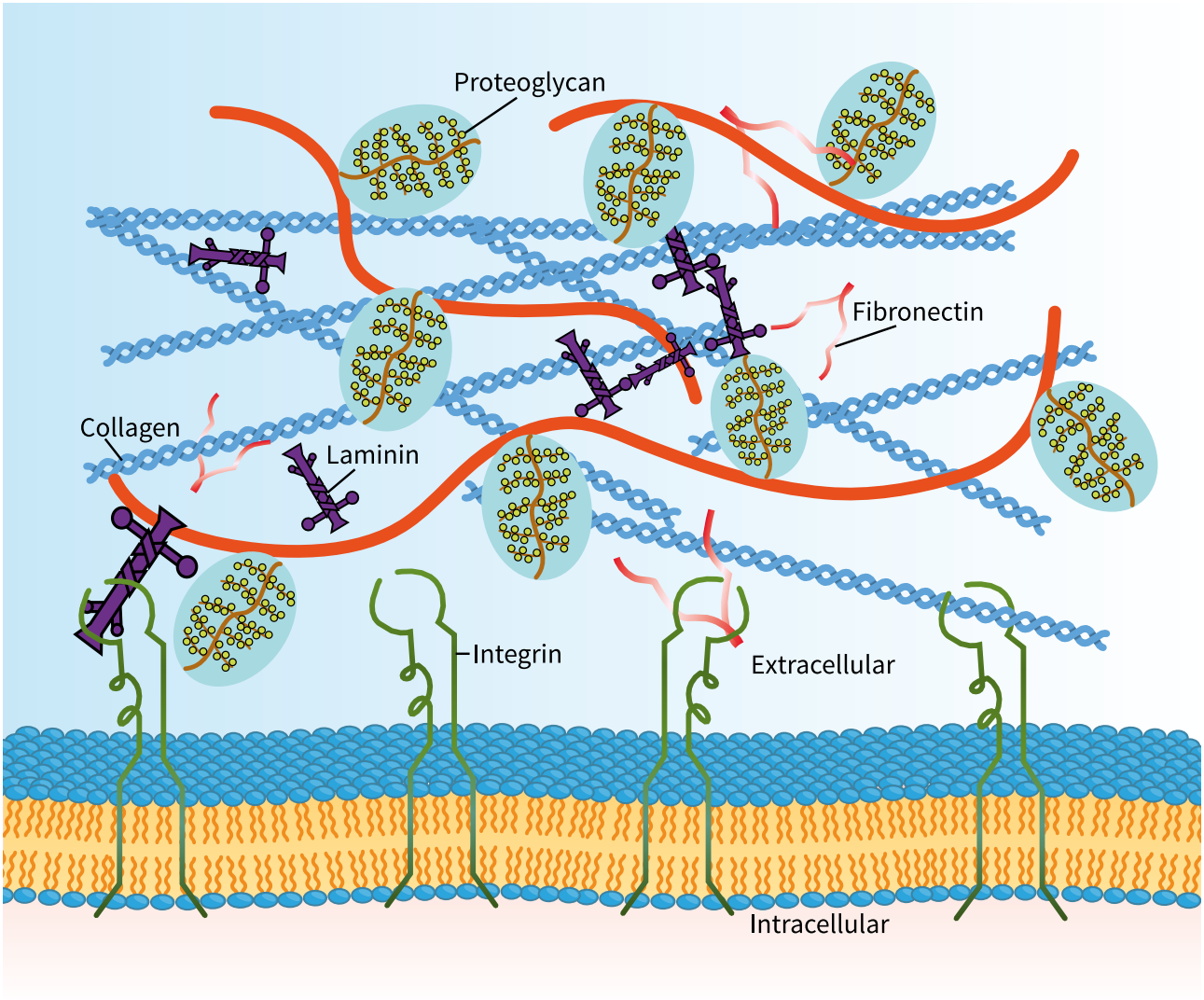


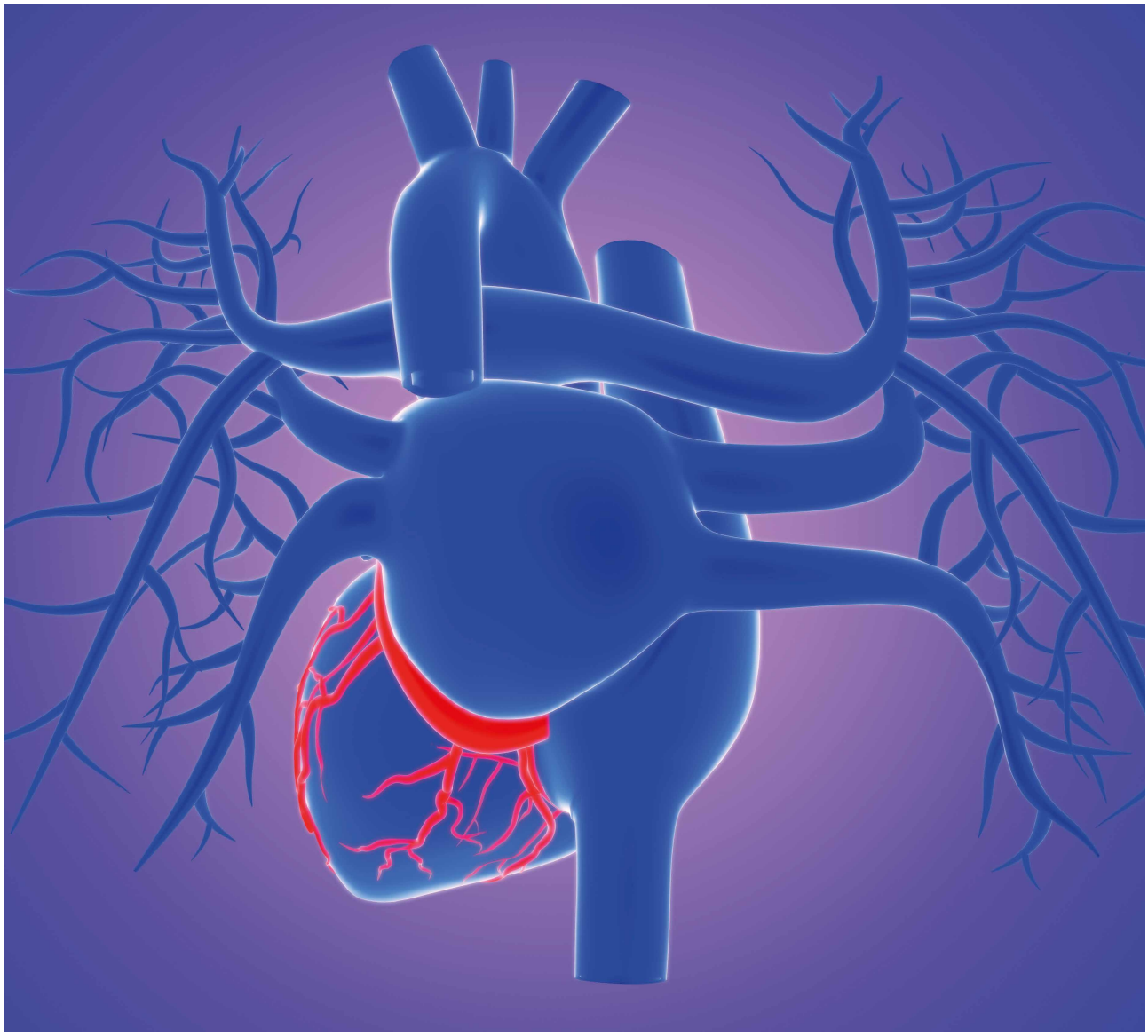
Illustration of the extracellular matrix – after the cell, this is the next important tissue component that can be targeted by different imaging techniques.

interactions between cells including signal transduction between them, cell migration, growth and cell death, as well as immunological functions. It contributes to the regulation of pH as well as hydration.

When tissues are inflamed, injured or invaded by tumours, the ECM is involved in an adaptive response, remodelling itself in defence. This remodelling involves changes in the ECM's biochemical composition and physiomechanical properties (i.e., the rigidity or elasticity of the scaffold). Due to the changes the ECM undergoes in diseased tissue, it is of interest as a target for *in vivo* (in the body) imaging approaches for the detection, characterisation and monitoring of disease. The CRC focuses on novel imaging techniques for the characterisation of the ECM.

The main structural components of the ECM include collagen, elastin and glycoproteins/proteoglycans. Collagen is a component of the skin, cartilage, tendons, ligaments, and bones and does not stretch much. When damage occurs as a result of inflammation or other trauma, the amount of collagen in the tissue can increase and results in fibrosis and scar formation, leading to a decrease in tissue elasticity. The protein elastin is a main component of the blood vessel walls. Tissue inflammation can lead to changes in the content of elastin in the ECM.

Proteoglycans (PGs) are a major component of the ECM. PGs consist of a core protein associated with carbohydrate groups consisting of glycosaminoglycans (GAGs). GAGs have a strong negative charge, which allows them to bind water and exert an influence on tissue properties. In different pathological processes, including inflammation and tumour invasion, the amount of one or more of the different types of GAGs in the ECM can be increased. An important characteristic of the GAGs is their ability to form complexes with positively charged molecules.



Goals of the CRC

Inflammation and fibrosis occur in different diseases, such as atherosclerosis (artery plaques), heart disease, multiple sclerosis, and inflammatory conditions of the intestine and liver. In all these diseases, changes in the ECM occur. The long-term goal for the CRC is the development of new imaging techniques for the characterisation of the ECM.

In heart disease, diabetes and hypertension, damage to the heart leads to remodelling of the ECM, which includes the increased formation of PGs and GAGs in order to regulate inflammation, fibrosis, and new blood vessel formation. An increase in collagen affects the biomechanical properties of the heart muscle, leading to a more rigid tissue.

The ECM also plays a role in the central nervous system, maintaining the structural integrity of the tissue through its interactions with various nerve and inflammatory cells.

In Crohn's disease, GAGs and collagen can increase in inflamed sections of the bowel wall, leading to fibrosis accompanied by fibrosis of the bowel wall.

In the development of liver fibrosis and cirrhosis of the liver, the composition of the ECM becomes impaired at an early stage due to a change in the synthesis and degradation of ECM components. The collagen content rises with the degree of fibrosis, resulting in an increase in tissue rigidity. There is also a distinct increase in a number of types of GAGs found in the fibrotic liver.

The researchers at the CRC 1340 include experts in the fields of diagnostic imaging, medical technology, nanotechnology, cardiovascular disease, neurology, and internal medicine. The group will investigate new imaging approaches for the imaging-based characterisation of the ECM.

Meet the researcher

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The Matrix in Vision Collaborative Research Center (CRC 1340) is a branch of the German Research Foundation consisting of a collaboration of 27 principal researchers from the Technical University of Berlin, Max-Planck Institute of Colloids and Interfaces, the National Metrology Institute of Germany, and the National Institute for Material Research and Testing. With a combined expertise encompassing many fields across physics, cell biology and biochemistry, the researchers share the common goal of investigating how different extracellular matrix components can be targeted for *in vivo* imaging using inflammation as a disease model. By experimenting with in vitro and *in vivo* model systems, combining molecular methods in radiology with new insights into how mechanical tissue parameters affect the development of disease, the CRC will investigate new molecular imaging probes and imaging approaches for a variety of clinically relevant inflammatory diseases.

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FUNDING

German Research Foundation (DFG)

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SFB 1309: COLLABORATING TO UNDERSTAND THE CHEMICAL BIOLOGY OF EPIGENETIC MODIFICATIONS

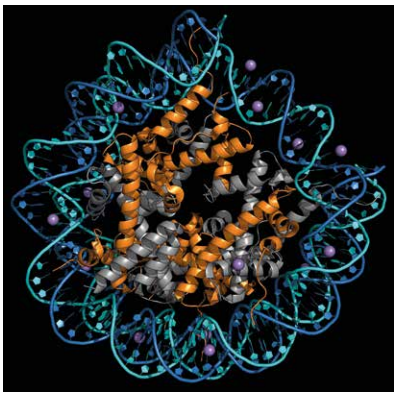
The discovery of DNA has been one of the most important findings of the last century, yet there is still much more to uncover about the 'additional chemical layer' brought about by the chemical modification of amino acids and nucleotide bases. A large collaboration of researchers at institutions across Germany, known as SFB 1309, is led by **Professor Thomas Carell** at the Ludwig-Maximilians University in Munich. A key focus of SFB 1309 is to elucidate the details of the second layer of information beyond the sequence layer of biochemical molecules called Watson-Crick bases within our DNA.

Exploring the World of Epigenetic Modifications

Following the success of the Human Genome Project which ran from 1990 to 2003, genetic mutations have been the primary focus for many researchers for several decades. While the permanent alterations in the DNA sequence caused by mutations have been important in creating the diversity of species we see today, these mutations also have a negative effect on human health. The research and development of novel approaches into these permanent changes in our genetic sequence have revolutionised the way we think of diseases such as cystic fibrosis, sickle-cell anaemia, and breast cancer. However, an additional layer of information lies beyond the genetic sequence, which has captured the attention of scientists and is responsible for encoding epigenetic modification.

At the DNA level, epigenetic modifications change the way how genes are expressed. However, unlike mutations, these modifications do not bring about any change to the genetic sequence itself. These changes occur by the addition of chemical groups to the nucleotide bases. Epigenetic modifications are reversible changes and the process of epigenetics is thought to be passed down through generations. The processes of epigenetics are necessary for normal body function, but chemical modifications to the genes and proteins can also result in damage, which may initiate disease.

Epigenetic modifications are important in determining which genes are turned on or off during development – also known as gene regulation. These modifications produce a layer of information that appears to be even more complex and diverse than the sequence code. This field is largely



unexplored but has the potential to lead to the future discovery of important therapeutic targets and novel breakthrough treatments targeting various diseases.

SFB 1309 is a large-scale collaboration of researchers at institutions across Germany, dedicated to advancing knowledge in this important field. Led by Professor Thomas Carell at the Ludwig-Maximilians University in Munich, the specific aim of SFB 1309 is

‘These studies inform us that our genetic system is chemically more complex than thought so far.’

to elucidate the modification processes acting on nucleic acids and proteins in order to better understand the properties and functions of these new biological molecules.

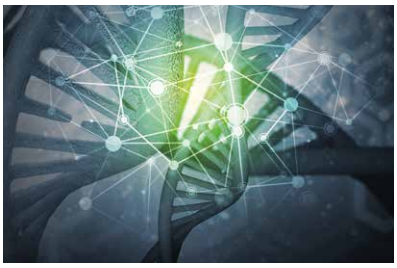
SFB 1309 has brought together renowned researchers across various scientific specialities in a collaborative effort to translate the chemical language that will uncover the mystery behind epigenetic modification. To this end, the collaborative team has split the research programme into three main areas of work. Area A will investigate the structure of DNA and RNA modifications. Area B will examine the functions of the modified nucleic acids and proteins. Finally, Area C will concentrate on the development of new techniques and tools to further develop this field of work.

Write, Read, and Erase

Epigenetic mechanisms are a normal part of human development. This is why even though cells generally contain the same genetic instructions from our genome, the concerted expression of our genes determines the differences in the structure and function of each of the hundreds of different cell types in the human body. Epigenetic regulators determine when these genes should be expressed.

The enzymes responsible for dealing with epigenetic information are characterised into three main types, namely, ‘writer’, ‘reader’ and ‘eraser’ proteins. Epigenetic writer proteins catalyse the addition of a chemical group on to the biomolecule, also known as epigenetic mark. Epigenetic erasers are a group of enzymes responsible for removing the chemical group, and epigenetic readers usually have specific elements able to bind to the epigenetic mark, converting the chemical message into biological action.

Unfortunately, errors in epigenetic modifications may result in changes to the regulation of gene expression.



This can be hazardous in cases where a protein responsible for activating cell growth may be produced in excess, which may lead to a cell dividing far more frequently than is normal – potentially leading to the development of cancer.

Currently, there is a lack of knowledge about the process that determines which nucleic acid is targeted and the reason why the modification needs to be inserted. However, the SFB 1309 research team will explore this specific issue. Investigation of the modifications requires the unravelling of the corresponding writer, reader, and eraser proteins. ‘We will decode the chemical code above the sequence

information and we will generate molecules that can interfere with the biological processes associated with reading, writing, and erasing this code,' explains Professor Carell.

Writer, reader, and eraser proteins are at the heart of understanding epigenetic mechanisms because they play a pivotal role in triggering biological processes. The SFB 1309 research team aims to develop chemical entities that will interfere with the biological processes of these proteins. In order to understand their functional implications, the researchers must also decode the epigenetic sequence to identify the position of the chemical modifications on the DNA and RNA molecules.

Professor Carell and the SFB 1309 collaborators plan to examine the writer, reader, and eraser proteins using modern mass spectrometry techniques to identify their structure and function. Using new sequencing tools, the team aims to determine the positions of the modifications on DNA and RNA sequences. Cell biology analysis will demonstrate the biological role of the modification and examine the chemical mechanisms that induce the enzyme's ability to modify proteins and nucleic acids. The team will drive forward the development of new proteomics and sequencing methods that will also determine the position of the modified units.

Unravelling the Layer Beyond DNA

DNA and RNA are nucleic acids consisting of a phosphate, a sugar and a base. DNA contains the sugar deoxyribose and RNA contains the sugar ribose. Five organic bases are involved in the formation of DNA and RNA – cytosine, guanine, adenine, thymidine, and uracil, which bind together to form the sequence code.

You may be asking now how the second layer of information in the genetic code was initially identified. To answer this, we must examine the previous work of researchers in the field. Scientists had initially discovered that additional DNA bases with a different chemical group existed in higher numbers, particularly in the neurons and stem cells of organisms with a high rate of reproduction.

The additional bases are 5-methyl-deoxycytidine (mdC), 5-hydroxymethyl-deoxycytidine (hmdC), 5-formyldeoxycytidine (fdC) and 5-carboxy-deoxycytidine (cadC), which are classed as modifications to the cytosine base. This discovery posed the question as to whether these four additional bases should also be considered naturally occurring DNA bases. However, at that time, detecting the chemical modifications was difficult due to the lack of technological development in the field.

Scientists later suggested that the modified cytosine bases hmdC and fdC were sites of damage to the DNA structure. However, it was discovered that an enzyme known as 'Tet' produced hmdC and fdC and these cytosine derivatives were located in specific areas of the genome, suggesting these molecules were sequestered for a specific purpose within the cell. Scientists have shown that also a large number of proteins bind to hmdC and fdC, suggesting that there is still more to know about these modified bases than previously thought.

As Professor Carell explains, 'These studies inform us that our genetic system is chemically more complex than thought so far.' The roles of these modified DNA bases will be investigated by the SFB 1309 collaborative research team, with a view to understanding their biological function, as well as detailing the underlying mechanism of the regulation of the Tet enzymes.

The SFB 1309 team also plans to synthesise modified bases and add them into a DNA sequence to investigate how their structures change. Identification of these structural changes will help to reveal the function of the chemical modifications.

Mass spectrometry is a very sensitive analytical technique that can detect the identity of molecules in very small amounts within the organism being tested. The team plans to use mass spectrometry to identify yet undiscovered modified bases. With recent advancements in scientific technology, modern mass spectrometers are now able to detect biomolecules at much lower levels than permitted by previous methods.

Unravelling the Layer Beyond RNA

As previously noted, the nucleotide bases within the DNA structure can undergo modifications naturally. When chemical groups alter the bases, this changes how the sequence is interpreted and, therefore, alters its properties and functions. The same occurs in the RNA sequence which codes for proteins, where post-transcriptional modifications influence how the genetic code is translated into an amino acid sequence. The range of modified nucleotide bases in RNA is far more diverse than that of DNA.

It is well established that both transfer RNA (tRNA) that is responsible for transporting amino acids, and ribosomal RNA (rRNA) that catalyses the synthesis of proteins, contain large levels of modified bases. However, scientists were surprised to find that also mRNA has considerably more modified nucleotides than they had predicted.

N6-methyl-adenosine (m^6A) is the most prevalent modified nucleotide base in mRNA. A lot of attention has been paid to the modified base, which is an adenosine derivative, which was one of the first modified RNA bases to be identified. Our current understanding of m^6A is that its formation involves an enzyme named methyltransferase, which acts as a writer protein, and directs itself to the adenosine base to attach a small functional group known as a methyl group. This plays an important role in regulating mRNA stability.

However, α-ketoglutarate dependent enzymes such as ALKBH5 and FTO remove the methyl group, thus behaving like eraser proteins. Scientists have demonstrated that the enzymes can remove the modification from the nucleotide bases and thus suggest that m^6A has a key role in cell function. The SFB 1309 research team aims to further investigate the enzymes involved. Professor Carell notes that 'It is … speculated that m^6A serves to destabilise mRNA sequences.' The m^6A eraser FTO has been linked to fat mass and obesity, and m^6A may be linked to nutrition and health, again demonstrating the importance of this second layer of information. Research into understanding how m^6A is regulated and its role in cellular processes is currently underway.

Although many of the modified bases have been discovered, scientists are yet to uncover the biological function for the majority of these modified biomolecules. Pseudouridine is another modified nucleoside in RNA – it was first identified in tRNA and later found in mRNA as well. Sequencing data for pseudouridine in mRNA is available, however, the reader and eraser proteins have not been identified so its function is as yet unknown. Professor Carell and the collaborative team aim to identify the protein responsible for writing, editing, and erasing modifications to help reveal the function of pseudouridine in this second layer.

Scientists have also discovered relatively recently a modified base known as ac^4C that is formed by an enzyme known as acetyltransferase NAT10. The modified base ac^4C is a derivative of cytidine and plays a role in maintaining the stability of mRNA and to improve the success of translation. However, the reader and eraser proteins involved in the modification of this base are also yet to be uncovered, and this is a further aim for the SFB 1309 research team.

The SFB1309 research team is also in the process of using synthetic bases to investigate the structure of RNA. Similar to the investigation into DNA, these structures will be used to reveal the function of the modification of RNA bases.

Strategies for the Future

Non-coding RNA (ncRNA) are RNA molecules that do not encode proteins but are important regulators in gene expression. The SFB 1309 research team is working to identify any modification that exists in ncRNA because of their importance in modulating gene expression and altering cell-signalling pathways. Professor Carell explains, 'It is currently thought that modified bases exist in ncRNA as well, but due to the difficulty in separating the ncRNA species from other RNA, this question is difficult to address. It is, however, now – with the new mass spectrometers that are available – the right time to investigate the chemical diversity of mRNA and ncRNA in more detail.'

In the post-genomic era, attention is being focused on the modifications that are important in cell division because understanding these complex pathways can help to develop important therapeutics in areas such as cancer. PolyADP ribose is a nucleic acid but it is also an important protein-modifying unit. PolyADP ribose forms a polymer and due to its similarity to DNA and RNA, it is involved in many different cell functions such as DNA repair.

A protein was recently discovered that binds specifically to polyADP ribose and regulates chromatin enzymes, which are important for protecting DNA from damage during cell division. The importance the modifier polyADP appears to have during cell division suggests that it may change the way cells are programmed to behave.

Professor Carell states 'Despite the fact polyADP ribose is named the third nucleic acid in contemporary biochemical textbooks, the interplay between polyADP ribosylation (the addition of ADP-ribose to a protein) and cellular plasticity awaits clarification.' To this end, the SFB 1309 research team will investigate the links between DNA damage and post-translational modifications of nucleic acids.

More research is needed to investigate the critical links between metabolism and epigenetic control. Scientists are now suggesting that the activity of mitochondria, the powerhouse of the cell, could play an important role in the control of the epigenetic modification mechanism.

In order to identify the role of the proteins that are involved in the modification of nucleic acid, the SFB 1309 research team will use inhibitors that are able to bind specifically to the modifying proteins. Inhibitors work by attaching themselves to the protein to disrupt their biological function. The team will design and synthesise these inhibitors so that they are able to observe the effect of turning off the mechanisms of these proteins.

Many human diseases are thought to have a genetic component, but the associated epigenetic mechanisms are yet to be identified. Professor Carell and his team are dedicated to uncovering critical new insights into the modified biomolecules and develop new methods to delve deeper into the phenomenon of epigenetics. As Professor Carell explains, 'We expect that understanding this layer of chemical information on biomolecules will allow us to find new avenues to treat diseases such as cancer and Angst-diseases such as schizophrenia and depression.'

In conclusion, the collaborative research conducted through SFB 1309 has the potential to provide ground-breaking insight into the true complexity of the second layer of the sequence code and open up exciting possibilities for epigenetic therapy for disease.

Meet the researcher

Collaborative Research Centre 1309

Chemical Biology of Epigenetic Modifications

Ludwig-Maximilians-Universität München

München

Germany

The spokesperson for the Collaborative Research Centre 1309 is Professor Thomas Carell. Professor Carell received his PhD in chemistry from the University of Münster in Germany and then completed a postdoctoral fellowship in Cambridge, USA. He became a university lecturer in organic chemistry at ETH Zurich, Switzerland, in 1995. He commenced a professorship in organic chemistry at the Philipps-University in Marburg, Germany, in 2000, then took up a professorship at Ludwig-Maximilians-University München, also in Germany, in 2003. During this time, he has received many awards and prizes, such as the Gottfried Wilhelm Leibniz-Award of the Deutsche Forschungsgemeinschaft in 2004 and the Otto-Bayer-Prize from the Bayer-Schering Foundation in 2008.

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STREAMLINING THE MODERN SUPPLY CHAIN

The digital age has seen a profound shift in how we consume products. Not only have many of us shifted to doing most of our shopping online; new technologies are also transforming how products are produced. These shifts have brought about similarly monumental changes to the supply chains that bring products to the doorstep, presenting significant new challenges to the complex networks of groups involved. In his research, **Dr Guoqing Zhang** at the University of Windsor in Canada uses the latest computational techniques and optimisation algorithms to present smart solutions to these issues.

Profound Change

In little over two decades, the ways in which most people in the developed world buy and consume products has changed almost beyond recognition. Previously, our only option to shop was to visit a physical store, whereas now, most of us frequently go online to buy products ranging from food to the latest technologies. Throughout the COVID-19 pandemic, online shopping has allowed millions of people to obtain essential supplies while remaining in the safety of their homes, helping to curb the spread of the virus.

In the US, the convenience of online shopping meant that total sales reached almost $350 billion in 2015 – a figure which was then predicted to grow annually by 6% until 2020. This profound shift spurred many companies to migrate from physical retail alone, to an approach that combines both physical and online channels.

Although this has largely occurred behind the scenes, such a significant transformation in behaviour has had a large influence on product supply chains, which encompass broad networks of people, companies, activities, information, and resources. Throughout their operation, these systems take in raw materials; transform them into finished products over successive stages; and finally, deliver them to consumers. This intricate process can be severely disrupted if any changes are not implemented effectively. In turn, this has required a substantial rethink amongst industries about how the movement and storage of materials and final products should be managed.

The Modern-age Newsboy Problem

Although this is a distinctly modern problem, Dr Guoqing Zhang at the University of Windsor shows that links can be drawn with the far older 'newsboy problem', which considers how managers need to make decisions about their inventory over limited periods.

In the past, news vendors faced constant uncertainty in the demand for their products. To deal with this problem, they needed to make smart decisions about how many papers to buy from a supplier each morning: if they bought too many, their leftover products would be worthless the next day; but if they bought too few, they would miss out on crucial opportunities to make more profit.

For modern industries, this problem becomes highly relevant when considering how multiple products should be sold – a situation that has now been widely studied for decades. In a 2008 study, Dr Zhang developed an algorithm that offered new solutions to this problem, accounting for factors including budget constraints, which limit the number of products that can be sold, and promotional price discounts, which would diminish profits if not enough products are sold. Since then, Dr Zhang has expanded on these advanced algorithms and mathematical techniques to explore how solutions to the newsboy problem can inform the supply chains of the digital age.

Recently, he also used a newsvendor model to formulate some supply chain risk management problems, and analysed the impacts of a manufacturer

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or retailer's risk attitude (such as risk-neutral or risk-averse) on ordering and marketing strategies. His team then developed an optimal capacity strategy for new product development with risk consideration, and applied it to the automotive industry.

Closed-loop Supply Chains

In a 2013 study, Dr Zhang became the first to consider how the newsboy problem is simultaneously affected by both supplier discounts and budget constraints: a situation that required an entirely new approach to analysis compared to previous approaches to the newsboy problem. His proposed algorithm was extremely effective in solving the problem on both small and large scales, even when it was extended to consider multiple realistic constraints. In particular, he considered 'closed-loop' supply chains – which involve products and materials travelling both forwards and backwards among networks of actors.

In this research, Dr Zhang investigated the characteristics of such a system made up of multiple interconnected plants, collection centres, demand-generating markets, and products. His methods led to a model that minimises the total costs for these supply chains, and that could be extended to consider external factors leading to unexpected variations in their operation. In addition, it could account for the impact of uncertainties in demand along both directions in the chain. To achieve this, Dr Zhang needed to incorporate elements of randomness into his models – a factor that would be crucial in his subsequent research.

More recently, his team studied production quantity, pricing and collection network decisions for manufacturing enterprises under either the 'take-back' or 'carbon emission capacity' regulations, and applied it to electronic waste collection and a tyre closed-loop supply chain.

Handling Two Channels

The dual physical and online channels described by Dr Zhang have brought about significant new challenges to the operation of modern supply chains. Together, they need to handle customers who can be distributed across wide geographical regions; deal with large volumes of orders that are often very small; and achieve short, flexible delivery times, often at night. As in the original newsboy problem, variations in demand across both of these channels can create significant uncertainties regarding how they should be operated.

Following on from his previous findings, Dr Zhang next aimed to analyse the impact of this type of commerce and 'omni-channel' commerce (referring to retailers with both a physical and digital presence to provide seamless shopping experience) on the management and logistics of supply chains, and to provide concrete solutions to how they should be configured effectively. In doing this, he used his algorithms to design new approaches to dual- or omni-channel commerce with random demand, which consider factors including facility locations, inventories, warehouse storage, and transportation. Through this approach, he hoped that his findings would provide key guidance to retail and manufacturing industries as they shift their operations to incorporate dual-channel supply chains.

Managing Warehouses and Inventory

Through a subsequent study, published in 2018, Dr Zhang drew on these cutting-edge techniques to suggest how optimised supply chains could be specifically designed to account for modern behaviours. To do this, he examined the inventory policies for newly emerging dual-channel warehouses, which are uniquely divided into two areas: one for fulfilling online orders; and the other for both storing products and fulfilling offline orders.

Dr Zhang also studied warehouse layout problems where both horizontal and vertical travel costs need to be considered, and developed several algorithms to solve these complex issues. Motivated by a real-world case, he also proposed an integrated strategy to combine production planning and storage layout, and analyse the impact of the 'Internet of Things' (IoT) on warehouse operations, in the first research to combine these two problems. His strategy ensures the availability of warehouse space, and saves costs during production and warehouse operation.

Realising Smart Supply Chains

Since the time of Dr Zhang's earlier research, an almost bewildering array of new technologies have emerged, including the internet of things, cloud

computing, and artificial intelligence. Although these terms can encompass widely varying systems, they are united in their ability to bridge the gap between advanced computational architectures, and everyday situations in the real world. Therefore, each of these technologies can be integrated into 'smart' supply chains to make them more cost-effective, share information more effectively amongst networks of companies, and better account for random uncertainties in the external environment.

However, these supply chains inevitably bring new challenges due to the manufacturing and business models they entail, and the high risks of uncertainty involved with applying new technologies. Therefore, it is critical to study how smart supply chains can be made to connect networks of companies and individuals with relevant technologies, and how industries can reconfigure and optimise their existing networks. Ideally, these systems would even have the capacity to intelligently plan, control, and adjust the systems to match supplies with ever shifting demands in real time.

Developing Optimisation Algorithms

To realise these capabilities, Dr Zhang and his colleagues are now working towards the development of advanced optimisation algorithms – which can find the best possible solutions out of all possibilities. This has involved exploring a variety of techniques and case studies. In his earlier research, Dr Zhang developed a software package for solving large-scale linear optimisation problems, which can be applied to various fields of study. Leading on from this, his team used these techniques to determine how the operation of a world-leading airline's vast cargo network could be optimised. From analysis of real data, the researchers solved routing problems, which would have once taken over 450 hours of calculation, in just 7 minutes.

More recently, Dr Zhang developed novel optimisation algorithms for solving diverse problems, including healthcare supply network issues and supply chain problems, such as warehouse layout and operation. Through their optimisation algorithms, his team showed how the operation costs of complex warehouses could be reduced by 26%.

Incorporating New Technologies

In his upcoming research, Dr Zhang will rigorously study the challenges faced by the supply chains of the digital age, resulting in new frameworks and optimisation algorithms for smart supply chains. In particular, he will focus on four key areas. Firstly, he will study the impacts and opportunities for new digital technologies like the internet of things and cloud computing, and will design new business models that can incorporate them effectively. Secondly, he will develop new concepts and methods for smart supply chain manufacturing – focusing on problems as wide ranging as uncertainty, tactical planning decisions, procurement, distribution, sales, and smart warehouse operation.

Thirdly, Dr Zhang will develop data-driven optimisation models, methods, and algorithms, which will enable him to study how modern data analysis can be combined with classical optimisation techniques to solve complex management problems. Finally, he will work with partners in real industries, including car manufacturing, energy, retailing, food, and medicine, enabling him to effectively solve the problems which arise for real-world supply chains, and to verify the solutions which have already been proposed.

Recently, Dr Zhang has also proposed an IoT-assisted randomised storage layout policy, and developed an integrated strategy to combine this with production planning. Through rigorous analysis, the IoT-assisted randomised storage policy can reduce operation costs by up to 28% and increase space utilisation by up to 17%.

Ensuring Success in Future Supply Chains

Through over a decade of research into how modern supply chains have been transformed by the digital age, Dr Zhang has made important advances in our understanding of how industrial practices have adapted. In the coming years, the technical landscape now looks set to transform in more profound ways than ever before – meaning his results have never been more relevant. By fully integrating the latest technologies and optimisation algorithms into the systems we rely on to bring products to consumers, while fully accounting for rapid improvements, Dr Zhang's methods could soon ensure that modern companies will not fall behind.

Meet the researcher

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Dr Guoqing Zhang achieved his PhD in Management Sciences from City University of Hong Kong in 2000. He has since worked at a variety of institutions worldwide, including as a postdoctoral fellow at McMaster University in Canada, a visiting associate professor at the University of Pittsburgh in the US, and a JSPS fellow at Osaka University in Japan. He joined the University of Windsor in 2002 as an Assistant Professor, then became an Associate Professor in 2005, and has been a Full Professor since 2011. His current research interests include smart supply chain and logistics management, the design of intelligent decision support systems, and optimisation algorithm design and development. He has earned numerous awards for his important findings in these areas, including the first practice prize of Canadian Operational Research Society in 2015 and the Outstanding Paper Award at the IEEM International Conference in 2014. He has also developed an effective optimisation software for solving large-scale linear programming.

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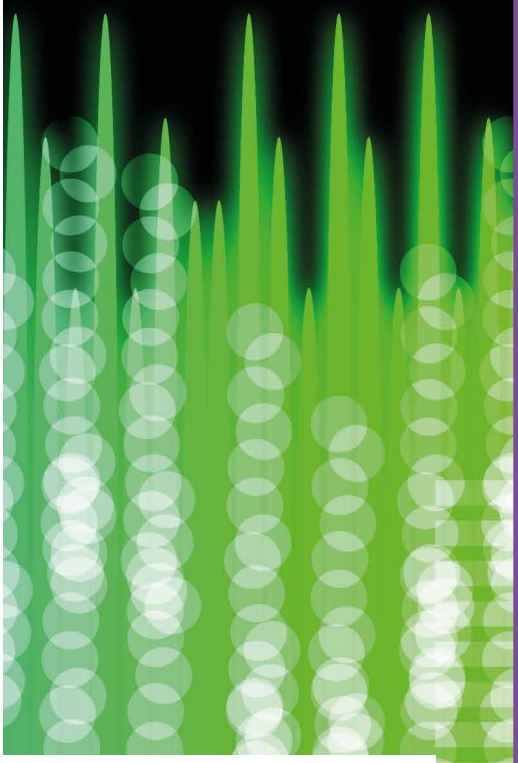
MUSICAL ALARMS: IMPROVING MEDICAL ENVIRONMENTS BY STUDYING SOUND



Incessant 'beeps' from medical devices are an all-too-common feature of hospital environments. Whether monitoring patient vital signs or warning of imminent emergencies, they form an integral part of modern clinical practice. For historical reasons, these devices generally communicate using simplistic beeps. This creates significant problems for both clinicians (who can struggle to differentiate the messages) and patients (who find them annoying and frustrating). **Dr Michael Schutz** from McMaster University is applying insights from musicians' use of sound to improve the quality of the auditory signals used in the life saving devices filling hospitals around the world.

Auditory Alarms in the Medical Setting

Our senses offer a path to understanding the world around us. We often use our eyes to connect with technology using visual interfaces – such as computer screens. However, in some situations, auditory interfaces can be more effective. For example, medical devices in hospitals use auditory interfaces to keep doctors and nurses updated while keeping their eyes focused on patients (which is crucial when engaged in complex procedures, such as placing a breathing tube).

Although these signals warn clinicians of serious medical emergencies, often they serve to communicate more mundane information. For example, the steady beep of a pulse oximeter provides updates on a patient's blood oxygenation saturation every time their heart beats.

Although they are crucial, designers of these sophisticated machines have traditionally overlooked the importance of the sounds used in their auditory alarms. The lack of sophistication in these tones render them annoying and distracting, posing risks for patient care and harming communication amongst medical staff. This is unfortunate and unnecessary, as many low-priority alarms offer useful information that is not immediately critical yet are designed using sounds that immediately grab attention. Unfortunately, since different devices use similar 'beeps', lower priority alarms can easily mask higher priority alarms, preventing doctors from getting crucial information in a timely manner. Furthermore, the annoying beeps interrupt patients' sleep, which is known to extend recovery times.

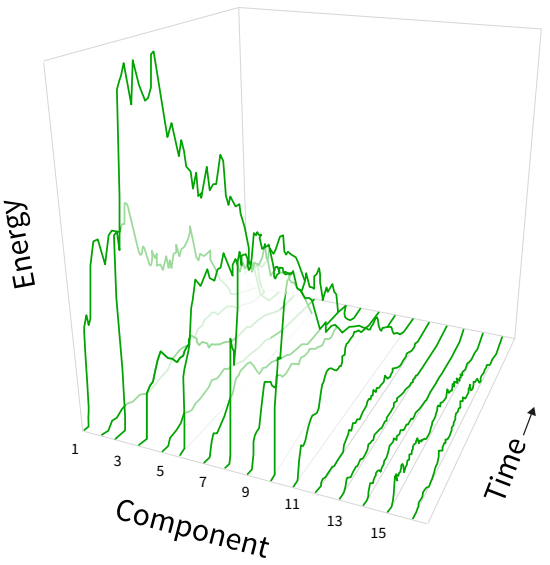
Dr Michael Schutz is working to apply knowledge from analysing music to propose new paths towards developing better sounds for these devices. He carries out his work at McMaster University in Ontario, Canada, where he founded a specialised laboratory

to support his team's research. In the Music, Acoustics, Perception & LEarning (MAPLE) lab, he mentors students making important discoveries on better approaches to structuring auditory alarms in medical devices.

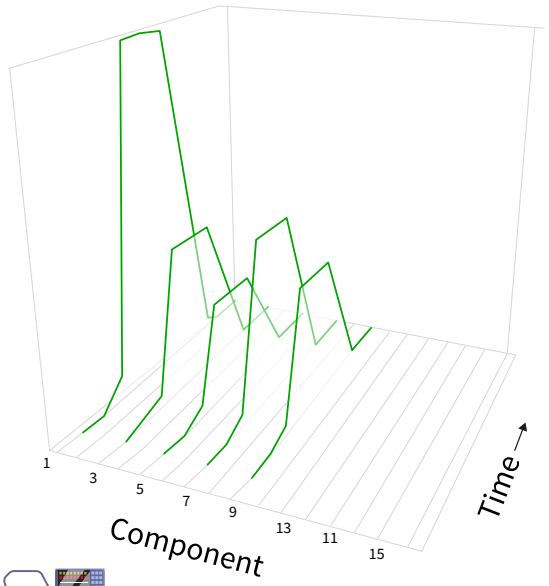
A Meandering Musical Path Leads to Medical Insight

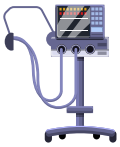
In one of his first papers published in 2007, Dr Schutz investigated how visual information changes how we 'hear' music. He showed videos of a world-renowned percussionist attempting to play long and short notes on the marimba, a large, xylophone-like instrument. He recorded the performer using physically long and short striking

‘Through my network of collaborators, my team is now well positioned to build on our auditory perception research to improve the sonic landscape of hospitals.’



Violin





Standard beeps

gestures, and ‘switched’ the sight and sound for half the trials. Participants rated their perception of the note’s length, unaware that some had been swapped.

Differences in the performer’s gesture failed to alter the resulting notes’ acoustic duration. Yet intriguingly, the gesture presented with the note changed the way participants heard the same acoustic information. This shows that watching the percussionist’s movement causes us to ‘hear’ the note differently. Apparently, the renowned musician’s long and short gestures failed to change the *sound* of his notes. Nonetheless, his gestures accidentally changed the way his notes *sounded* to audiences.

This finding of visual influence on the auditory evaluations of sound duration broke sharply with previous scientific inquiries. Dr Schutz subsequently documented that the reason for this discrepancy traces back to the complex nature of musical sounds – which contrast markedly with the simplistic tone beeps commonly used in auditory research.

The Structure of Sound

Sounds can be described in part through their ‘amplitude envelope’. This technical term refers to a sound’s ‘shape’ over time. Most natural sounds, including musical sounds, have complex shapes. For example, percussive sounds caused by impacts start quickly and begin decaying immediately. We typically find these dynamic sounds pleasing, and they are used frequently in music, for example, by instruments such as the piano, plucked harp and strummed guitar. In contrast, machine-produced beeps use markedly different shapes – including rapid offsets. This makes them very different from sounds heard in everyday listening as well as in music.

From an artistic perspective, a sound’s complexity plays a crucial role in the musical property of timbre. Timbre is what makes instruments sound different from one another, even when playing the same melody – a sort of ‘acoustic fingerprint’. The individual components of both complex music sounds (left) and standard machine beeps (right) are shown above. This type of 3D representation illustrates each component, or harmonic, on the horizontal (x) axis. Additionally, it shows the way each component’s energy (vertical, y axis) changes over time (z axis). In musical instruments such as the violin, the strength of individual components varies continually over time, with each component moving somewhat independently (left image). Yet in many medical devices the components are relatively constant, and typically start and stop at the same time (right).

Real-life Applications

Further work by Dr Schutz has shown that greater attention to the importance of sound complexity is not only important for scientists studying listening, but also offers practical applications such as enhancing our perception of product value.

In 2019, Dr Schutz published a paper demonstrating that people were willing to pay 9% more for products using

'Dr Schutz's work on music-inspired alarm design shows the benefits of careful attention to acoustical details, building bridges between functionality and aesthetics.' Dr Özcan Vieira, Director of Critical Alarms Lab at Delft University of Technology and Erasmus Medical Centre Rotterdam.

temporally complex (i.e., percussive-like wine glasses clinking) vs. simplistic (i.e., standard beep) sounds. Participants in that study received descriptions and pictures of two mobile phones and then heard sounds indicating a missed call. One of the phones used a percussive tone and the other standard beeps. Overall, 85% of people preferred the phone with the percussive tone, deemed it to be better quality, and would pay more for it. This illustrates the literal value-added as a result of better sound design – a crucial yet often under-appreciated aspect of product development.

For the moment, however, Dr Schutz and his team are focussing on applying their research to medical devices. Specifically, they are exploring how the use of sounds derived from music can be more effective at communicating with medical teams, while also being less irksome to patients and staff alike.

Many alarms follow the global medical standard set by the International Electrotechnical Commission, where sequences are made with three or five notes, all of which are based on standard beeps. Their simplistic shape requires them to be very short to avoid overlap. What might happen if each individual tone used a complex shape instead? This more complex approach would allow for the blending of notes into a sequence more similar to how music is structured. Dr Schutz's team took this approach in their next study, conducted in collaboration with anaesthesiologist and intensivist Dr Joseph Schlesinger of Vanderbilt University Medical Center.

Participants in that study heard an alarm along with its indicated issue (e.g., temperature problems). They then responded to the alarm's function when hearing it again, receiving feedback on their response. After a break to distract, they heard the alarm and again reported its function. Participants in this study were divided into two groups in which half heard alarms built on percussive tones and half heard alarms built on standard beep tones. Analysis of the responses indicates the type of sound used doesn't affect participant recognition or learning, but it does have one crucial impact – the new approach using percussive tones is resoundingly less annoying.

This study holds exciting promise for real-world application. Alarms based on percussive sounds are just as effective at communicating as standard beeps. However, as they are less annoying, they could provide a positive adjustment to the environments of those being treated, recovering and working in medical facilities. Given the ubiquity of these medical device alarms used in hospitals around the world, even small changes like reducing annoyance hold significant potential to improve public health, offering benefits for both patients and medical professionals.

Using Novel Software to Explore Complex Sounds

The MAPLE Lab team has built a new software tool called MAESTRO, which is now freely available at www.maplelab.net/maestro. Originally designed for Dr Schutz's teaching, his team now uses this software to generate sounds for their experiments as well. This tool eases the process of creating complex sounds, which is as useful in improving music cognition pedagogy as it is in assisting with crucial new experiments exploring the role of sound complexity in perception.

Drawing on his training and experience as both a professional musician and experimental psychologist, Dr Schutz bridges the traditional gap between music perception and music performance. His ongoing participation in music making through activities such as directing the McMaster Percussion Ensemble and performing at percussion festivals and music camps gives him a useful artistic perspective on the power of sound to communicate.

This insight, combined with his scientific training in conducting auditory experiments, has led to numerous discoveries that challenge conventional thinking about auditory perception, some with important practical applications for medicine and engineering. As Dr Elif Özcan Vieira, Director of Critical Alarms Lab at Delft University of Technology and Erasmus Medical Centre Rotterdam notes, 'Dr Schutz's work on music-inspired alarm design shows the benefits of careful attention to acoustical details, building bridges between functionality and aesthetics.'

Dr Schutz concludes by explaining 'Through my network of collaborators, my team is now well positions to build on our auditory perception research to improve the sonic landscape of hospitals'. This could both increase safety and decrease risk for patients and health professionals alike. His recent TEDx talk on this subject is now available at www.maplelab.net/ted and an interview with CHCH-TV is available at https://www.chch.com/hospital-sound-check/.

Meet the researcher

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Dr Michael Schutz received his Bachelor of Science in Computer Science, as well as his Bachelor of Musical Arts in Percussion Performance from Pennsylvania State University. He also holds a Masters degree in Music Technology and Percussion Performance from Northwestern University, and an MA as well as a PhD in Psychology from the University of Virginia where he worked with Dr Michael Kubovy. He is currently an Associate Professor of Music Cognition/Percussion at McMaster University in Ontario, Canada, and serves as a core member of the McMaster Institute for Music and the Mind. As founding director of the MAPLE lab, Dr Schutz supervises a diverse portfolio of research projects carried out with his interdisciplinary team, exploring the psychological basis of music and how this knowledge can be applied to a broad range of scientific and artistic issues. He also directs the McMaster Percussion Ensemble and serves on percussion faculty at the Honors Music Institute in State College, Pennsylvania.

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