Scientia

BREAKTHROUGHS IN BIOSCIENCE

EXCLUSIVES:

- Biotechnology and Biological Sciences Research Council
- Small Business Association

HIGHLIGHTS:

- A Novel Assay for a Novel Therapeutic
- Searching for New Biomarkers of Endometriosis
- Vicks Vaporub Shows its Speed
- How Mutant Staph Jumped from Livestock to Humans

Issue: #106

WELCOME...

Our healthcare continues to be transformed at an accelerating pace due to breakthroughs in bioscience research. Even in the past 50 years, our life expectancy worldwide has skyrocketed – people born this year can expect to live on average around 15 years longer than those born in 1966. We are enjoying healthier, longer lives as a direct result of scientific advances in vaccines, diagnostics, pharmaceutical and non-pharmaceutical treatments, surgery, nutrition and medical technologies such as drug delivery, medical imaging and radiotherapy.

In this edition of Scientia, we pay homage to the scientists behind our ever increasing quality of life by showcasing the latest in biomedical and biological research from around the world. To open the edition, we have had the pleasure of speaking with Professor Melanie Welham, the Interim Chief Executive of the Biotechnology and Biological Sciences Research Council, in an exclusive interview about the BBSRC's activities in supporting and promoting biological sciences in the UK and further afield. From here, we dive into the flourishing field of biomedical technology, where we highlight the latest in stem cell technologies and biomedical devices. In this section we also feature an interview with Nagesh Rao – Chief Technologist, and Chris McNeal – Presidential Management Fellow of of the Small Business Administration (SBA), who describe the ways in which the SBA's SBIR and STTR programs help small companies commercialise innovative biomedical technologies.

Throughout the issue we highlight the immense importance of medical diagnostics and nutrition to our health and wellbeing, along with how scientific advances in both pharmaceutical and non-pharmaceutical healthcare continue to improve our lives. Here we feature projects ranging from the search for biomarkers of endometriosis to the investigating the 'healthiness' of different fats in our diet, and helping children overcome the symptoms of ADHD using sensory-motor integration training. However, our innovations in medicine have not come without a price – in this edition we also discuss the rise of the super-bug, and feature three research projects dedicated to understanding and defeating drug resistance in bacteria. To close this edition of Scientia, we celebrate the continuity of bioscience research, by featuring three training programs, called *CaRTT, PEL* and *Choose Development!*, each designed to prepare the next generation of bioscientists for careers in research.

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Meet The Team...

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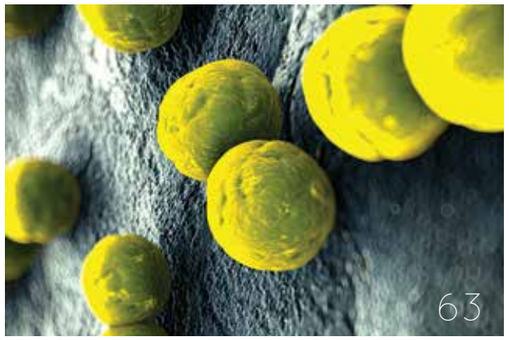


THE BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL

An exclusive interview with Professor Melanie Welham, BBSRC's Interim Chief Executive.

The Biotechnology and Biological Sciences Research Council (BBSRC) is one of seven Research Councils that work together as Research Councils UK (RCUK). BBSRC invests in world-class bioscience research, with the goal of tackling major challenges such as lessening the impact of climate change, achieving a healthier old age, and making our food and energy production sustainable. **Professor Melanie Welham,** BBSRC's Interim Chief Executive, gives us insight into the organisation, and describes the ways in which it supports and promotes biological sciences in the UK and further afield.

Firstly, could you please briefly introduce BBSRC, and tell us a little about its history, focus and scope?

BBSRC was formed in 1994 and for over 20 years has been investing in biological and biotechnology-focused research and innovation. Our Royal Charter defines three key objectives for BBSRC: to promote and support bioscience research and post-graduate training, to advance knowledge and technology to meet the needs of users for economic and societal benefit and to promote dissemination of knowledge and public understanding of biological science research. The bioscience we invest in spans plants, animals, microbes and humans, and we seek to fund the best research in the UK. One of our key aims is to support the UK bioscience research base so that it retains its world-leading status. What would you say are the guiding principles by which BBSRC is able to promote and support scientific research?

We are very clear – a key principle is to fund excellent bioscience research which has the potential, in the longer term, to have an impact – be this creation of new knowledge or translation of new discoveries so that they bring benefits to the people and economy of the UK.

What are the greatest challenges that currently shape the UK's research agenda?

BBSRC seeks to invest in excellent frontier bioscience across our remit. Within this, and in close consultation with experts across our research community, BBSRC has identified a number of grand challenges that form our strategic research priorities. These are: Agriculture and Food Security – producing sufficient safe and nutritious food to meet the demands of a growing global population using fewer resources; Industrial Biotechnology and Bioenergy – seeking to harness the power of biological systems to generate renewable supplies of energy, materials and fine chemicals; Bioscience for Health – helping the UK population to live healthier lives for longer, and Exploiting New Ways of Working – enabling researchers in the UK to be early adopters and developers of new approaches and technologies, as we did in the early days of genome sequencing.

In order to address these challenges, how does BBSRC, along with the rest of RCUK, promote cooperation between researchers across different disciplines and from different institutions?

All of the Research Councils recognise the importance of interdisciplinary research and are proactive in supporting research across disciplines. We often partner with other Research Councils to promote cooperation, for example the establishment of six multidisciplinary Synthetic Biology Research Centres, jointly supported by BBSRC, EPSRC and MRC and the Global Food Security programme, which not only involves Research Councils but also other Government departments. We also have a cross-council funding agreement that facilitates cross-council support of research applications that come in to us in responsive mode competitions.

Does BBSRC support international collaboration? What funding is available to promote research that might not be entirely UK based?

BBSRC is a strong advocate for international collaboration and we work closely with a number of international partners to support collaboration. A variety of funding opportunities are available, from small-scale international partnering awards that enable scientific exchange through to jointly funded research programmes, such as Lead agency agreements with the US National Science Foundation, Science Foundation Ireland and the Brazilian agency FAPESP. The Newton Fund supports research which primarily benefits developing countries and provides funding for joint collaborative programmes with 15 countries on the DAC list. We are also partners in many European funding schemes.

What are the main routes by which UK research receives BBSRC funding?

We operate a number of schemes through which researchers in UK universities and institutes can apply for funding to support their research. Our largest scheme allows researchers to submit their best ideas for research to us and these can be in any area of our remit – it is very much bottom-up researcher-led. We also have funding calls in specific areas related to our strategic priorities of Agriculture and Food Security, Industrial Biotechnology and Bioenergy, Bioscience for Health and Exploiting New Ways of Working. Often we will work in partnership with others, including research councils (e.g. Synthetic Biology Research Centres with EPSRC and MRC), Innovate UK (e.g. Agritech and Industrial Biotechnology Catalysts) and Industry (e.g. Research Industry Clubs), as well as with International Partners.

Considering the great range, and variety, of biological and biotechnological research that is undertaken in this country, how does the BBSRC ensure that the available funds are best allocated?

We seek to invest in excellent research wherever it is located in the UK and expert peer review is critical to achieving this. We assess all applications submitted to us using expert reviewers from across the research community (national and international scientists) and assessment panels then meet to discuss the applications, determine those of highest priority for funding and make recommendations to BBSRC on which should be funded. Involvement of the scientific community in peer-review is essential.

In the past you have worked upon BBSRC funded projects. Can you tell you tell us how that period influenced your own research career?

I have been very fortunate to have received support from a number of different funders during my research career. The award of a BBSRC Research Development Fellowship (2003-2006) had one of the biggest influences on my career. In essence, this Fellowship allowed me to focus all of my efforts on research as it allowed my university to employ someone to cover my teaching and administrative duties. I

was able to shift the focus of my research from investigating the processes controlling the behaviour of white blood cells to understanding how signals controlled the characteristics and behaviour of stem cells – embryonic stem cells became a particular focus. As a consequence of this fellowship my research team became focused on stem cell biology, which led to involvement in a large-scale European project as well as working as part of a public-private-partnership research consortia seeking to generate functional liver cells from embryonic stem cells.

Please tell us a little about the BBSRC's position on public engagement and knowledge dissemination?

Public engagement and science communication are important and we encourage our researchers to talk openly about their research and findings, processes and implications. For science to be useful and valued it needs to be an integral part of society – this means that as scientists we have a responsibility not only to tell people about our work but also to discuss and listen to others' views on our work and aspirations.

You have personally been involved in BBSRC outreach activities. Can you please tell us about your views upon the value of direct engagement with UK universities and research institutes?

Engagement with UK Universities and research institutes is vital for a number of reasons. First, it provides an opportunity to learn about the outcomes of the investments we have made in different areas of research. Secondly, we listen to concerns from our community – it is important that we work together to make a strong bioscience research base in the UK. Third, community engagement helps Universities and Institutes understand the influences on BBSRC as an investor of public funds as this carries with it very important responsibilities.

Finally, can you please share your thoughts on the future of biology and biotechnology in the UK, and the ongoing role of the BBSRC in that future?

There has never been a more exciting time! UK bioscience is world-leading and we have a real opportunity to expand the contribution that the Bioeconomy makes to the UK, improving the wellbeing of society and benefitting the wider economy.

'A key principle is to fund excellent bioscience research which has the potential, in the longer term, to have an impact'

BBSRC bioscience for the future

w www.bbsrc.ac.uk/

BIOMEDICAL TECHNOLOGY – SUCCESS THROUGH INNOVATION

Biomedical technology involves applying the fruits of research and development to address specific biomedical problems or questions to improve patient health. In recent years, advances in biomedical technology have been rapid and exciting. Human health and life expectancy have been enhanced and extended, respectively, by the efforts of biomedical scientists and the clinicians who apply their developed technologies. This sector also involves cultivating, stimulating and supporting the small businesses that can provide a hot-bed of innovation and imagination, in their quest to bring their products to market. In this section we showcase the results of two research groups and provide an overview of the Small Business Administration, who provide financial support and guidance for small biomedical companies in the United States.

In the first article of this section we talk to Nagesh Rao and Chris McNeal of the Small Business Administration. The goal of the organisation is to provide support for Americans who want to start their own small business and they have been instrumental in supporting small biomedical companies to engage in research and development and bring their products to market. The association offer seed funding to help small businesses get off the ground and provide support and advice. Next, we feature the work of Dr Phil Gan, a surgeon based in Australia who has invented a medical device to make minimally invasive surgeries easier and less traumatic. This device is called the LiVac™, and can be used to move and manipulate the liver during surgery, without causing it any damage. Finally, we look at the research of Professor Rhodri Ceredig and his PhD student, Andreia Ribeiro, of the National University of Ireland, Galway, who have developed a new assay to rapidly measure the potency of mesenchymal stromal cells (MSCs). The developed assay is rapid and inexpensive compared to previous techniques, permitting for standardised quality control and assessment between different laboratories.

THE SMALL BUSINESS ADMINISTRATION

An exclusive interview with Nagesh Rao – Chief Technologist, and Chris McNeal – Presidential Management Fellow of the SBA.

Small businesses are the creators of jobs and the driving force behind the US economy. Recognising this truth, the US government established the Small Business Administration (SBA) in 1953 in order to help Americans start and develop their own small businesses. As part of this initiative, the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs support early stage businesses that wish to commercialise innovative biomedical technologies. Between them, these two programs awarded $2.5 billion dollars of seed-funding directly to small businesses last year alone. Over the next few pages we talk to **Nagesh Rao**, the Chief Technologist, and **Chris McNeal,** Presidential Management Fellow of the SBA, who give us an overview of the organisation, and describe the ways in which the SBIR and STTR programs help small companies engage in R&D, bring life-saving technologies to the market and create jobs.

To start, please briefly introduce the SBA, and tell us about its history and focus.

The Small Business Administration (SBA) is an independent federal agency created in 1953 to 'aid, counsel, assist and protect the interests of small business concerns, to preserve free competitive enterprise and to maintain and strengthen the overall economy of our nation'. The SBA helps Americans start, build, and grow businesses. The Agency utilises an extensive network of field offices and partnerships with public and private organisations to deliver services throughout the United States, Puerto Rico, US Virgin Islands and Guam.

Since our founding, the SBA has delivered millions of loans, loan guarantees, contracts, counselling sessions, and other forms of assistance to small businesses throughout the country. The Office of Investment and Innovation focuses on the Small Business Investment Companies (SBICs), Growth Accelerators, Small Business Innovation Research (SBIR), Small Business Technology Transfer (STTR), and Federal and State Technology Partnership (FAST) programs. Chris and I primarily focus on the SBIR and STTR programs, where the SBA serves as the coordinating agency for the eleven participating agencies. SBA directs the agencies' implementation of SBIR and STTR, reviews their progress, and reports annually to Congress on its operation.

In the midst of overhaul and revamping of SBIR.gov, among many other programmatic endeavours, we at SBA were fortunate to hire at the end of 2014, John Williams into a Senior Executive Service role as the Director of Innovation and Technology lead for policy and programmatic oversight of the program. He brings his experience and success from leading the Navy SBIR program to help our SBA team in building out successful efforts across the 11 agencies.

In what ways are the SBIR and STTR programs able to promote and support small businesses to engage in Research and Development?

SBIR targets the high-tech entrepreneurial sector because that is where most innovation and innovators thrive. However, the risk and expense of conducting serious R&D efforts are often beyond the means of many small businesses. By reserving a specific percentage of federal R&D funds for small businesses, SBIR protects the small business and enables it to compete on the same level as larger businesses. SBIR funds the critical start-up and development stages and it encourages the commercialisation of the technology, product or service, which in turn stimulates the US economy. Since its enactment in 1982, the SBIR program has helped thousands of small businesses to compete for federal R&D awards. Their contributions have enhanced the nation's defence, protected our environment, advanced health care, and improved our ability to manage information and manipulate data. SBIR's mission is to support scientific excellence and technological innovation through the investment of federal research funds in critical American priorities to build a strong national economy. Its goals are to stimulate technological innovation, meet federal R&D needs, foster and encourage participation in innovation and entrepreneurship by socially and economically disadvantaged persons, and to increase private-sector commercialisation of innovations derived from federal R&D funding.

The STTR program also expands funding opportunities in the federal R&D arena. Central to the program is expansion of the public/private sector partnership to include joint venture opportunities for small businesses and non-profit research institutions. The unique feature of the STTR program is the requirement for the small business to formally collaborate with a research institution in Phase I and Phase II. STTR's most important role is to bridge the gap between performance of basic science and commercialisation of resulting innovations.

The mission of the STTR program is to support scientific excellence and technological innovation through the investment of federal research funds in critical American priorities to build a strong national economy. Its goals are to stimulate technological innovation, foster technology transfer through cooperate R&D between small businesses and research institutions, and to increase private sector commercialisation of innovations from federal R&D. Iconic companies that have resulted from our program have included Qualcomm, Symantec, Biogen-Idec, iRobot, Intuitive Surgical, LiftLabs, Made in Space, Ecovative Design, Natel Energy, Oceanit and Orbital ATK.

What are the greatest challenges that currently shape the public/private sector partnership agenda?

Balancing the needs of the various stakeholders involved in small business companies, major corporations, and academic institutions to name a few. Each entity is going to see the path to success differently and will have different timelines as well end goals in mind. Thus it's critical to goal-align as much as possible and allow each stakeholder to do what it does best in helping shape a more positive future.

In order to address these challenges, how does the SBA promote co-operation between companies and researchers across different disciplines and from different institutions?

The SBIR and STTR programs are excellent examples of the SBA's efforts to promote cooperation between companies and researchers across different disciplines. Eleven agencies participate in the SBIR program and five of those eleven agencies also participate in the STTR program. Companies and research institutions are able to search for opportunities on our website (sbir.gov). It is not uncommon for the same company to compete for and win funding from multiple agencies

over the course of developing their product. There are also numerous examples, such as iRobot and Hydronalix, where companies sold their commercialised SBIR and STTR funded projects to multiple audiences.

The SBA also continues to look for ways to improve our outreach and engagement efforts to ensure that we are reaching underserved communities. We host a series of SBIR Road Tour stops across the country to convey the message of the program. Entrepreneurs can meet with program managers at these events to discuss the program, ask questions, and learn about funding opportunities. The SBIR program is hitting the Road this April to get the word out about the program across the country. Be sure to visit www.sbirroadtour.com for more details and to find out what city we will be coming too this year.

Do the SBIR or STTR programs support international collaboration? What funding is available to promote business that might not be entirely US based?

The SBIR and STTR programs are designed to award contracts and grants to US-based companies to drive innovation and commercialisation. The SBA can however connect small businesses with resource partners who specialise in international support functions. Our office has also met with foreign delegations to discuss how our programs operate and we make efforts to work with our sister agencies, International Trade Administration and US State Department to assist on helping US firms figure out appropriate global scale up opportunities.

Considering the great range, and variety, of research that is undertaken in the USA, how do the SBIR or STTR programs ensure that the available funds are best allocated?

Each agency sets the criteria for their proposed contracts and grants. The company or research institution must meet the qualifications of the proposal and compete for the funding. The SBA oversees the government-wide SBIR and STTR programs, but does not control which projects are awarded. SBA authorises award amounts above the statutory limits and sets the guidelines for the program government-wide. However, while the SBA does not control the allocation of funds, we know that the program is highly competitive. Approximately 15% of proposals are awarded Phase I funding, and only 25% of those proposals are awarded Phase II funding. We know that the proposals are high risk, and we have witnessed high rewards across multiple sectors. SBIR and STTR funded concepts drive innovation, and that spirit produces products that revolutionise our lives. Over the lifetime of the program we have invested around $40 billion into cutting edge high tech small business companies, and merely scratching the surface, Qualcomm and Biogen alone have a collective market cap of $120 billion essentially tripling the ROI of the program.

Please tell us a little about the SBA's position on public engagement and knowledge dissemination.

One of the critical aspects of our programs is that the entrepreneur retains the intellectual property rights to their ideas and that your data is protected. Data generated from the entrepreneur's federal Research or R&D is protected from public disclosure for a minimum of four years (civilian agencies) or five years (DoD) after the conclusion of the award. During this time, we encourage the entrepreneur to secure patents on their work. We want to preserve their intellectual property rights, which the small business company gets to retain per discretion of the Bayh-Dole act. Furthermore, we offer non-diluted funding (no equity taken).

The SBIR and STTR programs are designed to award contracts and grants to US-based companies to drive innovation and commercialisation.

SBIR·STTR America's Seed Fund

www.sba.gov
www.sbir.nih.gov
www.sbirroadtour.com
www.sbir.gov

We are constantly working on expanding the awareness of our programs. The SBA hosts a variety of SBIR Road Tour events, targeting underserved states, in addition to events such as our National SBIR/STTR Conference.

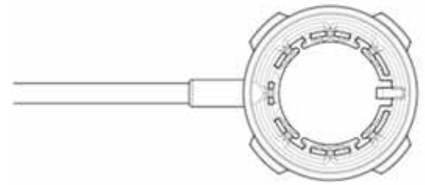
Please tell us about your views on the value of direct engagement with regards to small businesses collaborating with research institutions.

One unique feature about the STTR program is the requirement for small businesses to formally collaborate with a research institution during Phase I and Phase II. This partnership is essential to bridging the gap between basic science and commercialisation. In other words, helping to facilitate effective cooperation and technological de-risking by high tech small business firms portioning some of their STTR funding to a research institution to aid in the necessary R&D. SBIR and STTR are highly competitive programs that encourage domestic small businesses to engage in federal Research or R&D that has the potential for commercialisation. Through a competitive awards-based program, SBIR and STTR enables small businesses to explore their technological potential and provides the incentive to profit from commercialisation. Including qualified small businesses in the nation's R&D arena stimulates high-tech innovation and fosters entrepreneurial spirit while ensuring the government meets its specific research and development needs.

Finally, can you please share your thoughts on the future of research commercialisation, and the ongoing role of the SBA in that future?

SBA will continue to ensure that small businesses continue to have opportunities to drive research and development commercialisation. We are continuously improving our outreach and engagement efforts to ensure that the program reaches all corners and communities of our nation. We are currently working with our partners in Congress to reauthorize the SBIR and STTR programs. Last fiscal year, the SBIR and STTR programs provided over $2.5 billion dollars of seed-funding directly to small businesses nationwide. The SBA will continue to work closely with our sister agencies to ensure that these programs are top priorities across the federal government.

SUCKING THE LIVER OUT OF THE WAY WITH LIVAC™



Dr Phil Gan, a general surgeon, has invented a surgical device called the LiVac™ Retractor, that has significant advantages over traditional methods during laparoscopic surgery which require the liver to be retracted.

Any technological advances which reduce the amount of trauma caused by surgical procedures can enhance patient recovery and help to minimise complications. Laparoscopic, or 'keyhole' surgery, has allowed many procedures to be performed without the need for major incisions (laparotomy). Although this has proved a major advancement, surgeons are constantly trying to improve their techniques, such as by reducing the number and size of incisions required for a particular operation. Dr Philip Gan is a general surgeon based in Australia, who has made such an advancement through the invention of an innovative piece of technology called the LiVac™ Retractor.

Issues with retracting the liver

The liver, our largest internal organ, must often be retracted, or moved out of the way during laparoscopic operations to allow the surgeon access to particular parts of the body. The left lobe of the liver is retracted for surgery performed on the stomach, while the right lobe is retracted to allow access to the gallbladder and right kidney. A device called a Nathanson retractor, which has been in use for decades, consists of a curved and bended steel rod, is traditionally used for retracting the left lobe of the liver. The retractor is inserted through an incision in the epigastrium (the central region of the abdomen just below the rib cage) and placed under the liver before being fixed to a frame bolted to the operating table. Retractors may be held in place during the operation by a surgical assistant, who may also be operating the laparoscope. These methods require an incision and/or port specifically to insert the retractor.

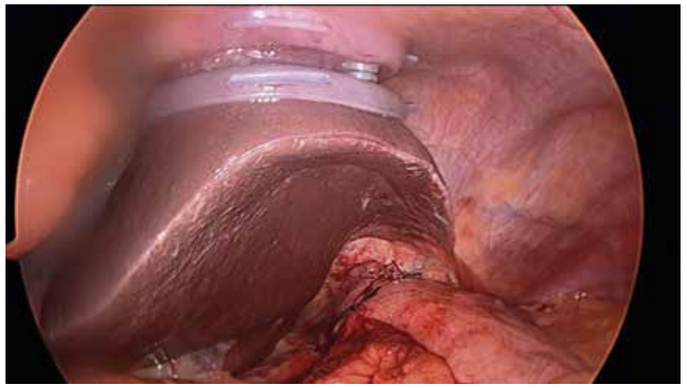
The pressure exerted by the retractor can congest the internal vasculature of the liver, leading to tissue trauma from disturbance of the blood supply and subsequent re-perfusion injury. This damage can occur in varying degrees of severity, ranging from mild tissue congestion all the way up to rare reports of liver necrosis and death. Increased time periods for which the tissue is compressed results in higher risk of liver injury, as well as a subsequent inflammatory response which makes the patient feel unwell.

Retraction techniques can also include suturing (stitching) of the liver or gall bladder to the abdominal wall, which may cause undesirable tissue trauma.

From ideas to prototypes

'In any field, if you are not moving forward, then you are going backwards. Innovation is the catchword of the moment, and not without good reason', says Dr Philip Gan. During surgery, Dr Gan observed the left lobe of a liver which had adhered to the diaphragm from surface tension alone, and saw an ingenious way to move the field of surgery forward. 'It occurred to me that if surface tension alone could hold up the left lobe of the liver, then so too could suction.' Over the next few years, his idea developed into the LiVac™ Retractor (Liver Vacuum), which after lodging a provisional patent in 2010, he produced with the help of an Australian medical device manufacturer and engineering company, Ingeneus Pty Ltd. Dr Anabela Correia, a specialist in the commercialisation of medical technology, and a number of consultants in the industry also assisted over the subsequent years.

‘The LiVac™ Retractor is the only retractor which attaches to the superior surface of the liver, whilst also being hands-free and not requiring any additional incisions.’



The device is a collapsible, soft, ring-shaped object made of silicone, attached to suction tubing. When attached to a regulated source of suction, a vacuum is created in the space inside the ring. The retractor is placed in apposition between the liver and the diaphragm, and suction is then applied to the LiVac™ Retractor. This then mimics the effect which Dr Gan had originally observed, in adhering the liver to the diaphragm. Compared with hand held retractors, the surgical assistant would instead be able to give their full attention to optimising the view through use of the laparoscope. No attachment to a fixed frame is required when using the LiVac™, since once the suction is applied, the diaphragm takes the weight of the liver, rather than its weight being taken by an attachment to external apparatus. The LiVac™ suction tubing can exit alongside existing ports and therefore a dedicated incision is also not required, as it would be when using the Nathanson retractor.

The LiVac™ Retractor is produced in two sizes, large (78mm diameter) and small (56mm), with the smaller version being suitable for retraction of the left lobe and the larger size being more suited to retraction of the right lobe. The collapsible nature of the LiVac™ Retractor means that incisions through which the retractor is inserted do not need to measure the entire width of the ring. In fact, retractors of either size can be inserted through the lumen of a 15mm port or the tract created by a 12mm port. The LiVac™ Retractor is also disposable, which is a desirable specification for most pieces of surgical equipment, as it guarantees sterility.

Trials and testing

Dr Gan and Ingeneus developed prototypes, and by 2012 the device was ready to be tested on laboratory animals at the University of Melbourne Veterinary School. The tests proved successful and the results were later published in the medical journal, *Surgical Endoscopy*. However, it can be difficult to extrapolate from animal tests into how a surgical device may perform in humans, due to the anatomical differences between the livers of humans and those of animals such as pigs and sheep. The next stage of testing therefore needed to be performed on human patients, which is a considerably more complex process than animal testing.

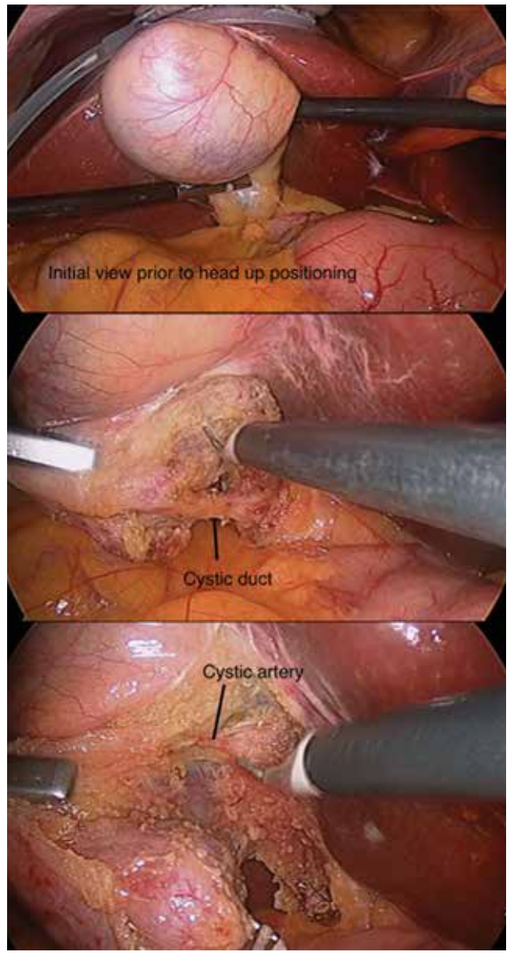
In 2013, Dr Gan’s team successfully applied to a highly competitive, match-funded Australian Federal Government grant program for commercialisation. With the money secured for an in-human trial, his team then addressed the legal, ethical and scientific requirements necessary for clinical testing of medical devices. Two committees, the national St John of God Healthcare Ethics Committee and the Ethics Committee of Southwest Healthcare approved the trials.

Impressively, the development of the trial protocol, patient recruitment, surgery and follow up were conducted within the grant’s short time-frame, which was less than one year. One three-port hiatus hernia repair with anterior fundoplication, three single-incision gall-bladder surgeries, three reduced-port (3) gall-bladder surgeries and three reduced-port (3) gastric band operations were performed. The term ‘reduced port’ refers to the smaller number of incisions which would normally be required to perform the operation. The patients’ recovery and any complications were carefully monitored and reviewed by independent trial coordinators, as well as the operations themselves being video recorded. No device-related adverse events were observed and all performance milestones were achieved. No bleeding, lacerations or tears were observed and livers displayed only embossing, which flattened a few minutes after removing the retractor, leaving only bruising.

The results were presented at several high-profile, international surgical conferences in 2014 and 2015 (the European Association for Endoscopic Surgery (EAES) 14th World Congress in Paris, June 2014, the 2nd International Consensus Conference on Laparoscopic Liver Resection (ICCLLR) in Iwate, Japan in October 2014 and the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) Congress in Vienna, Austria, in August 2015), and also published in Surgical Endoscopy.

Regulatory approval came in late 2014 with listing on the Australian Register of Therapeutic Goods (ARTG) and issuance of a European Conformity (CE Mark) certificate.

Dr Gan was pleased to receive a letter of congratulations from the current Premier of Victoria at the time, Dr Denis Napthine.

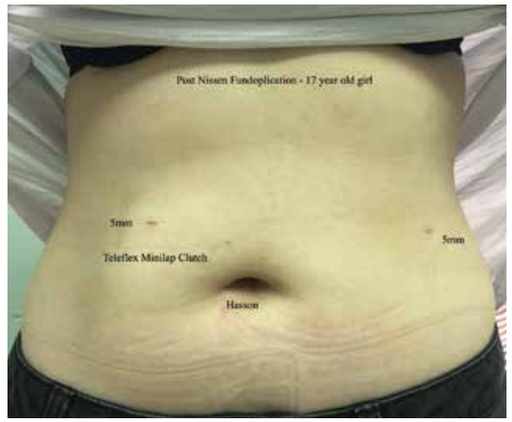


The LiVac™ retractor in practice

Dr Gan has subsequently used the LiVac™ Retractor as his standard retractor in gastric and gall-bladder surgeries. ‘I have used it on many different types of liver, including severely fatty livers, which are the ones most prone to retraction injuries as they are so soft and friable’, says Gan. He has also been able to directly observe the effect of the LiVac™ in patients who have, for one reason or another, had to undergo other laparoscopic procedures. One, for example, required an appendectomy six weeks after a laparoscopic three-port cholecystectomy (gall-bladder removal surgery) which involved liver retraction using the LiVac™ Retractor. No visible mark was seen on the patient’s liver. Similarly, none the other patients he has needed to re-laparoscope have shown any residual marks on the liver.

‘In any field, if you are not moving forward, then you are going backwards’

In two patients who required liver biopsies during other operations in which the LiVac™ Retractor was used, core biopsies were taken from retracted and non-retracted parts of the liver then sent to pathologists separately, blinding the pathologist as to where each core was taken from. The pathologist was unable to differentiate between the two samples in these two patients.



Dr Gan describes a laparoscopic Nissen Fundoplication, used to treat gastro-esophageal reflux disease, which was performed on a teenage girl, ‘using a combination of the LiVac™ retractor with a Hasson port, two 5mm ports and a needlescopic 2.3mm grasper, leading to very insignificant scarring, little pain and most importantly, a very happy patient.’

The future of LiVac™

The future of Dr Gan’s business appears bright, as patents have now been granted in Australia, New Zealand, the USA, Japan and China, and with patents pending in a number of other countries including the EU. The LiVac™ Retractor is still a new technology in the surgical field and awareness of this invention is just starting to grow. Using the LiVac Retractor does not change the fundamental techniques used during surgery. However, as with any new technology, there is a learning curve and support is available for surgeons interested in using the device.

The company’s priority is to introduce the LiVac™ Retractor to interested early adopters, innovative surgeons who share the goal of truly minimally invasive surgery. When these surgeons have built up their experience with the LiVac™ retractor, then it will be time to do a comparative clinical trial with the Nathanson Retractor. Surgeons themselves may also come up with ideas and applications that can be incorporated into future developments.

It is important for surgeons to have options to choose from when performing surgery, based on the anatomy of the individual patient, such as their BMI, liver size, shape and pathology. On this natural variability and importance of a range of options, Dr Gan says, ‘Laparoscopic surgery can be done in very different ways, from using multiple large calibre ports to few mini-laparoscopic incisions, and choosing the least invasive, yet safest, approach for a particular patient makes a difference.’ The LiVac™ Retractor increases the options available to surgeons by providing a retractor that does not require an additional incision, is hands free and is gentle on the liver.

Meet the researchers

Dr Philip Gan began studying medicine at the University of Melbourne in 1985, and became a Fellow of the Royal Australasian College of Surgeons in 2001. Dr Gan currently works as a consultant general surgeon at the St John of God and Southwest Healthcare Hospitals, Warrnambool, and the Portland District Hospital. He has a keen interest in minimally invasive laparoscopic surgery, including laparoscopic colorectal resections, hernia repairs, cholecystectomy, bariatric surgery, fundoplication and splenectomy (largest 2.6kg).

Dr Anabela Correia achieved her PhD in Medicine from Monash University and runs her own technology commercialisation business. She has over 10 years of experience in the field of commercialisation of medical technology and has worked as General Manager of Ipernica Ltd, senior consultant at PricewaterhouseCoopers and Commercial Business Development Manager at Monash Commercial.

Judy Bingham BPharm, FSHP, MHSc, GAICD. Judy Bingham is a registered pharmacist who worked in hospital pharmacy, medical affairs in the pharmaceutical industry and as a senior director for an international clinical research organisation before establishing her own consulting company, Easington. Judy now provides regulatory and clinical strategy, planning and management services, supporting medical technology companies to bring innovative new product to global markets

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A NOVEL ASSAY FOR A NOVEL THERAPEUTIC

Professor Rhodri Ceredig and his **PhD student Andreia Ribeiro** have developed a novel assay for measuring the immunosuppressive potency of MSC (mesenchymal stromal cells) that could revolutionise the use of this cell-based therapy

How did your research experience prior to this project lead to an interest in developing an assay to quantify MSC potency? How did you come to work together?

Rhodri Ceredig: The main topic of my research career has been to study the development of cells of the specific immune system, namely lymphocytes, in mice. My doctoral and post-doctoral research focused on the development of functional T cells in the embryonic and neonatal mouse thymus. After creating a transgenic mouse line that over-expressed interleukin-7 where animals routinely developed B/myeloid lymphomas I later became interested in B cells. To further study these cells, a colleague in my lab, Dr Amanda Fisher, generated a clone of bone marrow-derived MSC. Still interested in B cells, I joined the laboratory of Prof. Ton Rolink at the Basel Institute for Immunology; Rolink was using MSC-based culture systems to study B cell development. While at Basel I also met Prof. Alan Tyndall, a rheumatologist with interest in using MSC to treat autoimmune disease. I became interested in characterising MSC via flow cytometry and began collaborating with Tyndall. I came to Galway in 2008 as an immunologist to join Prof. Tim O'Brien's Regenerative Medicine Institute (REMEDI). In collaboration with Prof. Noel Lowndes, my primary focus is the understanding of the DNA damage response of MSC, particularly in the context of hypoxia. With my colleagues Prof. Thomas Ritter and Prof. Matthew Griffin, we answered a funding call from Science Foundation Ireland to investigate the potential of developing a novel flow cytometry based assay with which to measure MSC immunosuppression; this funding included financing for a technical assistant, and Andreia was chosen based on her past experience with MSC and flow cytometry. Andreia has continued this work as part of her PhD funded by a EU Marie Curie Skłodowska Initial Training Network.

Andreia Ribeiro: I worked in a flow cytometry diagnostic laboratory where I had the opportunity to study lymphocyte response to MSC isolated from different sources. I developed a strong interest in MSC and found myself with many unanswered questions at the end of the project. I applied to Prof. Ceredig's lab in the hope of pursuing some of those questions.

Why do we need an assay to quantify MSC potency?

Compared to our knowledge of blood cell development, our understanding of MSC is relatively crude. However, because of their differentiation and immunosuppressive properties, MSC show promise as a therapeutic modality for the treatment of autoimmune and/or degenerative diseases. Many experiments have demonstrated the immunosuppressive properties of MSC, yet their mechanism of action is unknown. Further, there is a glaring commercial and scientific need for a potency assay with which to test whether individual batches of MSC have equivalent immunosuppressive effects and more importantly, whether particular MSC batches will have immunosuppressive activity on a potential recipients' immune cells in a medical setting.

What impacts do you hope this MSC assay will have on medical research? Do you plan to extend this work further?

Rhodri Ceredig: We hope to see this assay used in clinical trials where MSC-based therapies are being attempted. Currently we do not have any correlation between results from our in vitro potency assay and in vivo clinical outcomes, but such clinical trials are being initiated in Galway. We await their outcome with anticipation.

I am coming to the end of my research career, during which I have been lucky to work alongside many talented, rigorous scientists. I came to Galway with the aim of passing on some of the experience I have gleaned from laboratories around the world to the next generation of scientists. I am excited to see upcoming scientists, such as Andreia, take up the mantle of MSC and immunology research.

Andreia Ribeiro: If clinical trials demonstrate a correlation between in vitro and in vivo results, this assay will ideally go on to be used as a tool to provide personalised medicine. It would be possible to predict if a certain batch of MSC would be beneficial to a particular patient ahead of treatment. While at the moment I am focused on finishing my PhD, I hope to have further opportunities to study MSC and immune cell interactions as I continue my career. MSC products have the potential to provide powerful therapeutic approaches for several conditions. I hope that I can contribute to this research in a small part; there is still so much work to do!

BRINGING MSC FROM THE LAB BENCH TO THE DOCTOR'S OFFICE

In recent years, research highlighting the potential for MSC as a powerful therapeutic has boomed. However, inconsistencies in production and quality control present a major barrier to cell based therapies entering mainstream medicine. **Professor Rhodri Ceredig** and **PhD student Andreia Ribeiro** recently developed a novel quantitative assay to test the potency of MSC strains.

Cell-based therapies: the future of medicine?

A cell-based therapy is a medical treatment during which live cells are injected into a patient to treat a disease or condition. These therapies hold the possibility of treating conditions ranging from cancer, to heart disease, to diabetes, with healing capabilities far beyond those of most traditional pharmaceuticals. Over the past decade, research in cell-based therapies has flourished, and a large number of cell-based treatments for a variety of conditions have shown great promise in clinical trials. However, very few of these potential therapies have made it through the processes of licensing and market authorisation necessary to make the leap from clinical trials to a product available to the general public. Because the product in these treatments are live cells, manufacturing is far more complex than a typical pharmaceutical, and reliable quality control measures present a formidable hurdle to widespread distribution of cell-based products. Without appropriate assays to assure the quality, safety, and potency of a cell-based treatment, these potentially powerful therapeutics may be doomed to stay in the laboratory.

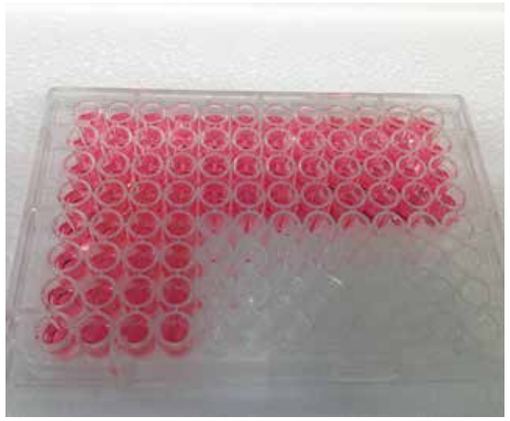
MSC: Promise & Problems

MSC (mesenchymal stromal cells) are multipotent cells that are capable of differentiating into many of the types of cells that form tissue, including bone, muscle, fat and cartilage cells. Researchers have discovered that MSC can act as powerful pharmaceuticals, working to stimulate healing in conditions that require tissue repair and as immunomodulators in inflammatory and immune conditions. These multi-talented cells have been shown to play a role in down-regulating inflammatory immune processes in autoimmune diseases, with the potential to improve acceptance of new tissue following an organ transplant. Human bone marrow-derived MSC demonstrate promise as a potential therapeutic for many diseases, and currently over 550 clinical trials involving MSC are in progress worldwide. While researchers are not yet entirely sure how MSC act to regulate immune function, these cells have shown consistent aptitude for therapeutic use in clinical trials and MSC research is booming.

Unfortunately, not all MSC are created equal in the lab. In addition to the many research institutions culturing MSC, numerous private companies have emerged with a focus on developing batches of MSC for commercial pharmaceutical purposes. There is no universal method for producing MSC, and individual facilities vary in their approaches, which may lead to differences in MSC quality and potency between sources. Even within the same facility, variation between bone marrow donors can produce widely different potencies between batches. This high potential for batch variation poses a difficult quality control problem for MSC production facilities, and is one of the greatest barriers to cell-based medical treatments making it to the general public. For MSC to advance as a viable therapeutic, a standardised potency assay that allows for the comparison of individual batches is needed. Further, as medical research advances, doctors are moving away from the 'one-size-fits-all' approach to treatment and turning to technologies that allow practitioners to take personalised medical strategies for individual patients. Because a patient may respond uniquely to MSC preparations from different sources, an assay that allowed practitioners to rapidly select the most effective MSC batch for an individual patient prior to treatment could be transformative in medicine.

Creating a novel assay

Until now, the only available procedure to test MSC potency took over 24 hours, is expensive, and requires both equipment and skilled technicians that are not common to smaller facilities and hospitals. Ceredig and Ribeiro sought to develop a practical, quantitative assay for MSC potency with potential for wide deployment across the variety of settings where MSC are used. The ideal assay should be fast, practical, inexpensive, and produce quantitative results that are comparable between assays. Additionally, an assay with the option to utilise a patient's own blood to test the effectiveness of multiple MSC batches would create opportunities for personalised medicine.



To achieve these goals, Ceredig and Ribeiro chose to exploit one of the best-described immune effects of MSC treatment, reduction in pro-inflammatory products from monocytes. Monocytes are a type of immune cell that operate as part of the innate immune system, the body's first line of defence in an infection and the component of the immune system implicated in inflammation. Monocytes are widely circulating in the blood, their responses to many kinds of stimuli are rapid and well-understood, and their immune products are well-characterised. When activated by compounds associated with pathogen presence, monocytes produce many pro-inflammatory products, including TNF-*α* (tumour necrosis factor alpha). TNF-*α* is a cell signalling protein with known involvement in many diseases, including arthritis, inflammatory bowel disease, and asthma. When monocytes are activated by bacterial endotoxin, TNF-*α* is produced rapidly in a dose-dependent manner, making it an excellent indicator of monocyte activity.

Given the ease of obtaining whole blood with monocytes and the quick and reliable production of TNF-*α* in response to commonly available endotoxin, TNF-*α* emerged as a clear target for an MSC potency assay. This choice was validated by demonstrating that TNF-*α* expression was reduced in a dose-dependent manner when MSC were added to the whole blood mix. By comparing TNF-*α* levels between bacterial endotoxin activated monocytes with and without a specific dose of MSC, it is possible to reliably evaluate how potent a batch of MSC is relative to other batches. Further, while different MSC batches performed similarly across volunteer blood samples, there was some variation in potency between individuals. This provides preliminary support for the use of this assay in personalised medicine, upcoming clinical trials will expand on these findings.

One of the critical components for the development of the potency assay was the availability of the BD Accuri C6™ bench-top flow cytometer with attached automatic sampler module. This two laser, four-color flow cytometer allows simultaneous detection of cell surface and intracellular proteins. In addition, the C6 sampler module allows automatic acquisition of samples from 96 well microtiter plates. Although not done routinely, the whole potency assay can be semi-automated in a 96 well format using a Perkin-Elmer Janus robot for the cell cultures and the BD Accuri C6™ cytometer for readout. In this manner, the assay has been used to screen compounds for immunosuppressive activity. The BD Accuri C6™ is an affordable, reliable, sensitive and easy to use flow cytometer that makes flow cytometry an accessible tool for any laboratory.

Why do we need an assay to quantify MSC potency?

Further, there is a glaring commercial and scientific need for a potency assay with which to test whether individual batches of MSC have equivalent immunosuppressive effects and more importantly, whether particular MSC batches will have immunosuppressive activity on a potential recipients' immune cells in a medical setting. Potency assays are available on the market (e.g. MSCGlo™-96 PRS), but these primarily deal with MSC growth and differentiation and not immunosuppressive activity.

Shaping the future of MSC in medicine

The novel MSC potency assay developed by Ceredig and Ribeiro provides a viable option for MSC quality control across research, manufacturing and clinical settings. This assay can be completed in a single day using widely available equipment and reagents, at a much lower cost than previous protocols. This assay will allow researchers to provide standardised measures of the potency of MSC lines used in their experiments. Companies producing MSC for commercial purposes will be better able to perform quality control on their MSC preparations and ensure potency of their products. From a therapeutic perspective, this assay could allow practitioners to select MSC preparations from multiple sources using a patient's own blood, opening the door to personalised medicine for a myriad of conditions that MSC show promise for treating.

The next steps in the development of this novel assay include clinical trials that correlate the in vitro results obtained in the lab to in vivo results in human patients. The assay has been patented, and if it reliably predicts how well patients will respond to MSC treatment in clinical trials, then there is a strong potential for its commercialisation and widespread distribution. This would be a major stride towards MSC therapies becoming widely available.

Meet the researchers

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Professor Rhodri Ceredig received his PhD from the Walter and Eliza Hall Institute of Medical Research, Victoria, Australia. Through his career he has studied the immunology and development of T cells, B cells, and MSC in prestigious laboratories around the world. He joined the Regenerative Medicine Institute at the National University of Ireland, Galway, in 2008 to pursue questions about the immunological properties of MSC and train graduate students in immunology research.

Andreia Ribeiro received her Master's degree in Microbiology from the University of Aveiro, Portugal, in 2010. She went on to join the Center of Histocompatibility in Coimbra, Portugal, where she worked to characterize mesenchymal stem cells from various sources. In 2011 she joined the laboratory of Rhodri Ceredig initially as technical assistant and now as a PhD student. The primary focus of her doctoral work is on the effect of hypoxia on MSC extracellular matrix.

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IMPROVING OUR DIAGNOSTICS

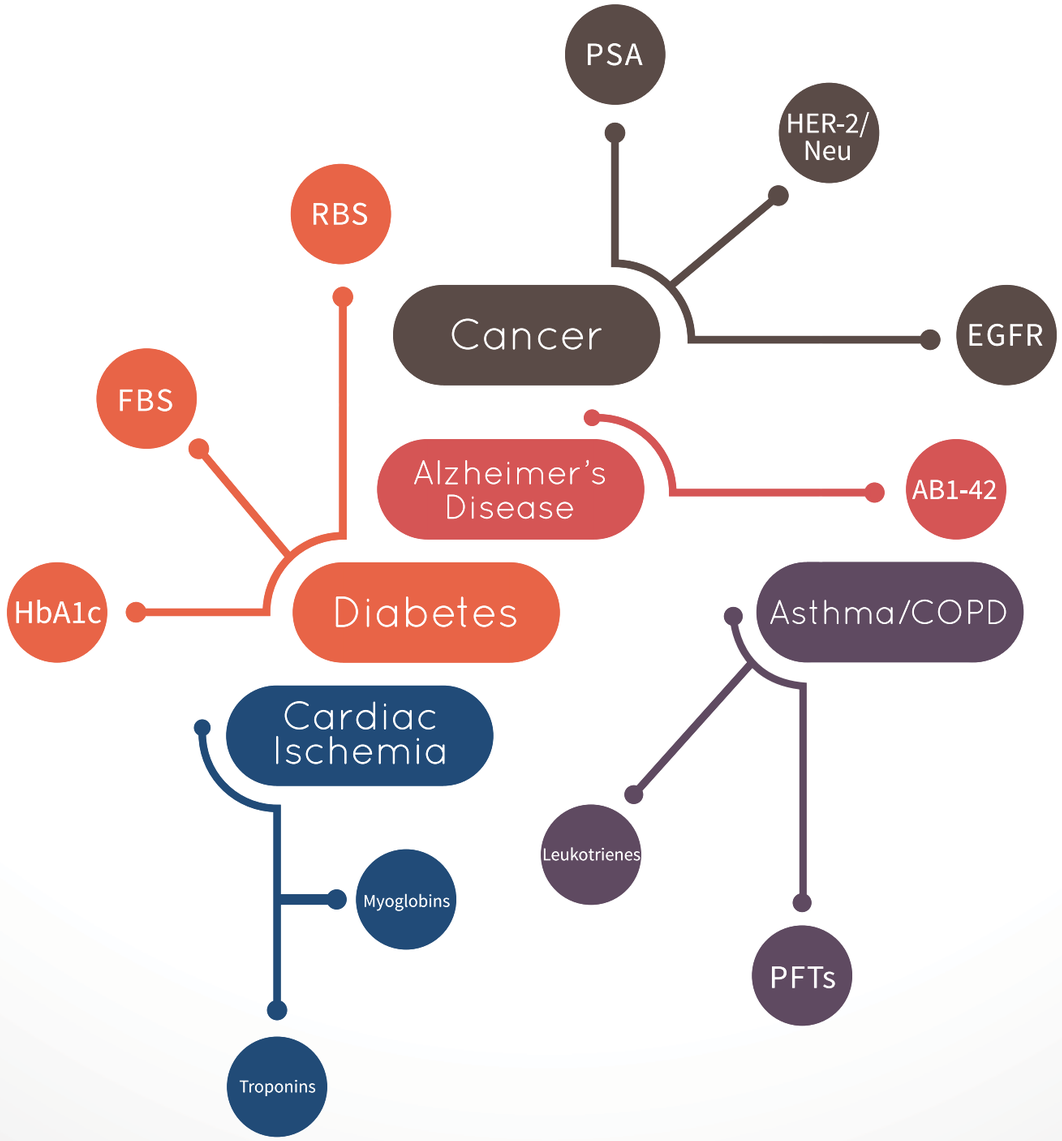
The ability to diagnose disease easily and efficiently is of utmost importance in healthcare. Fast diagnostic tests help to catch any problems that arise, before they develop into more serious conditions. For example, the emergence of cervical smear tests, which test for abnormalities in the cells of the cervix caused by the HPV virus, has dramatically reduced the incidence of cervical cancer amongst women in the past few decades. Upon detection, any precancerous lesions can be removed from the cervix, long before they have the chance to develop into life-threatening cancer.

A rapidly developing field in diagnostics research involves the search for previously undiscovered biomarkers. Biomarkers are typically compounds that can be isolated from blood, urine or other bodily fluids, and their occurrence can be used as an indicator of the physiological state of a patient. Many different biomarkers are currently used in the diagnosis of multiple diseases; for example, the level of troponin in a patient's blood can help to determine if their heart tissue has been damaged following heart attack, while elevated levels of amylase in the blood can indicate pancreatitis. In addition to being hugely important in preventive medicine, biomarkers are also seen as a route to personalised medicine – treatments individually tailored to individual patients for efficient intervention in disease processes.

In this section of the edition we introduce Professor Warren Foster and his collaborators at McMaster University, who are working to find biomarkers for endometriosis – a prevalent gynaecological disease that causes severe pelvic pain and infertility. Currently, the delay between the first onset of symptoms and an accurate diagnosis of endometriosis is estimated to be a staggering 8–10 years, during which time women suffer without receiving appropriate treatment to lessen the symptoms. The delayed diagnosis of this condition is due to the invasiveness and cost associated with the current method of diagnosis, which entails a laparoscopy. Therefore, the discovery of a strongly predictive and reliable biomarker for endometriosis would result in a less invasive diagnosis of the disease, meaning that women could be diagnosed much more easily, with a simple blood test for example. Such a biomarker could also potentially be used as a measurement of treatment success; whereby declining levels could indicate a successful treatment intervention.
Along the same thread, Dr Sharon Ruthstein at the University of Bar-Ilan in Israel is developing a technique to analyse copper biomarker signatures in tumour environments. Her fascinating research into the biochemistry of copper using electron paramagnetic resonance technology may revolutionise future diagnostic and therapeutic care for cancer patients. As we will see in this section, Dr Ruthstein's copper research also extends to Parkinson's and Alzheimer's disease, as both conditions are associated with elevated copper levels.

Biomarker research is currently in the process of being extended to the diagnosis of psychiatric disorders, but unfortunately due to the nature of mental illness, progress in this field is slow. As a result, physicians must often rely on psychological assessment in the diagnosis of many mental health conditions, rather than using biological diagnostic tools. Due to the problems associated with employing such a blunt instrument in the diagnosis of mental illness, many conditions go undiagnosed for year. For example, clinical recognition of bipolar disorder can often take as long as 10 years. Here is where the work of Dr Charles Bowden comes in. Along with his colleagues at the at the University of Texas Health Science Center, he has developed better assessment tools for bipolar disorder and for improving diagnostic criteria, using a new technique called the Multi-state Outcome Analysis of Treatments (MOAT) methodology. This innovative new approach has already successfully assessed the efficacy of the drug lamotrigine, in a clinical trial that has led to the drug's FDA approval for the treatment of bipolar disorder.

COMMON BIOMARKERS OF DISEASE



SEARCHING FOR NEW BIOMARKERS OF ENDOMETRIOSIS

Professor Warren Foster and his collaborators at McMaster University are interested in finding biomarkers for endometriosis, a gynaecological disease that causes pelvic pain and infertility. They hope that the discovery of a strongly predictive and reliable biomarker for endometriosis could result in a less invasive diagnosis of the disease, meaning that women could be diagnosed more easily. Such a biomarker could also potentially be used as a measurement of treatment success, whereby declining levels could indicate a successful treatment intervention.

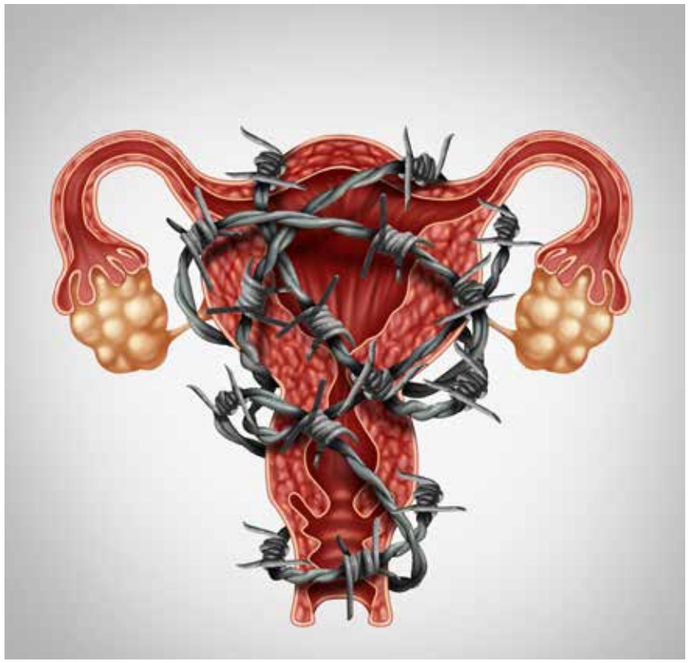
Endometriosis – what is it and how many women does it affect?

Endometriosis is a gynaecological disease, where specialised tissue, that under normal circumstances lines the interior of the uterus, grows in other locations outside the uterus. This tissue, called the endometrium, is a specialised layer that lines the interior of the uterus. Under normal circumstances, the function of the endometrium is to provide a surface for the implantation of a fertilised egg. Under the influence of female sex hormones, it grows and thickens to form a blood and nutrient-rich lining during the menstrual cycle, and functions as a permissive and nurturing environment for early embryonic development, should conception occur. The outermost layers of this tissue are routinely shed during the menstrual cycle, provided conception has not occurred. In endometriosis, fragments of this tissue arise outside of the uterus, in the abdominal cavity. This is called ectopic endometriotic growth (ectopic means in an abnormal position). This can result in pelvic pain, pain during sex and infertility. Depending on the location of the lesions, the disease can be classified as stages 1–4, which correspond to minimal, mild, moderate, and severe disease. The precise cause of endometriosis is unknown, but the disease is relatively prevalent. Approximately 10% of women of a reproductive age are affected by the disease, which applies across all ethnicities. In women with chronic pelvic pain, the prevalence increases to 30% and in infertile women up to 50% are affected. This translates to 176 million women worldwide who suffer from endometriosis and 5.5 million in the United States alone.

Societal impacts and current treatments

The societal impacts of this disease are numerous and significant. Expensive surgical procedures, drug treatments and lost time from work all contribute to a substantial economic cost. In the United States alone, the annual cost of treating endometriosis is estimated to be a staggering 22 billion

‘Our work is the combined effort from a group of very talented and dedicated investigators. Their experimental results have allowed us to build a really strong research program in endometriosis that has enabled us to make important contributions in defining suitable clinical markers of endometriosis.’



dollars, which surpasses expenditure on other chronic conditions such as asthma or Crohn’s disease. The cause of endometriosis is unknown and there is no known cure. Current treatments aim to manage the disease and provide relief from its symptoms. Surgical interventions to remove the endometriotic lesions from the abdominal cavity are often implemented and can provide a measure of relief from pelvic pain. A frequent aim of treatment is to preserve or enhance fertility, where possible. In some cases, a hysterectomy (complete removal of the uterus) is necessary, or feasible in women who do not wish to have children. Treatment with pain relieving drugs is also often required, but the long term use of such drugs can be associated with significant side effects. Hormonal treatments can be used to counteract or block the actions of estrogens, which helps to reduce the growth of the endometrium during the menstrual cycle. This can help to limit the negative effects of ectopic endometriotic growths on patient well being, but can also cause significant side-effects and can interfere with fertility and the chances of conception. Additionally, it is currently difficult to determine when the disease will ‘flare up’, causing pain and discomfort, and so hormonal therapies are often required to be used all the time, meaning that affected patients need to endure their side effects almost constantly and may not be able to conceive.

Current difficulties diagnosing endometriosis

One of the major challenges in the treatment and management of endometriosis lies with the difficulty in identifying it in a timely manner. The symptoms associated with endometriosis, such as pelvic pain can sometimes be confused with other conditions, which cause pain in this area, such as irritable bowel disease or pelvic inflammatory disease, and the use of oral contraceptive pills can also suppress some symptoms, confusing a potential diagnosis. In fact, the delay between the first onset of symptoms and an accurate diagnosis of endometriosis is estimated to be 8–10 years. This means that affected women have to endure the symptoms of endometriosis without appropriate treatment for this extended period. Additionally, the harmful inflammatory processes inherent to endometriosis proceed unchecked during this period, which can lead to neuropathic pain syndromes, which can be devastating for women who have exhausted present supports trying to get help. A major factor that contributes to the delayed diagnosis of endometriosis lies with the invasiveness and cost associated with the current gold standard method of diagnosis. This approach entails a laparoscopy, where a small incision is made in the abdominal wall and a fibre-optic camera is inserted to view the abdominal cavity and identify endometriotic lesions that are present. Not only is this procedure expensive, invasive and requires specially trained medical personnel, but it can sometimes be difficult to provide a clear diagnosis. Endometriotic lesions do not always look the same and patient-to-patient variability can make a conclusive diagnosis difficult. Additionally, this method is only of use in identifying larger lesions and microscopic disease may not be identified. Consequently, significant efforts have been devoted to identifying reliable biomarkers for endometriosis.

Biomarkers for endometriosis

Identifying biomarkers for endometriosis would greatly improve the ability of physicians to reliably diagnose the disease in a minimally invasive way, perhaps through a simple blood test, leading to fast track diagnosis and appropriate management. This is the motivation underlying the work undertaken by Professor Foster and his team. A variety of factors have been identified by different researchers as differentially expressed in patients with endometriosis compared with healthy controls, which is perhaps unsurprising given the inflammatory response present in such patients. However, to date, none of these factors has proven to be strongly predictive or sensitive enough to function as a robust and reliable biomarker.

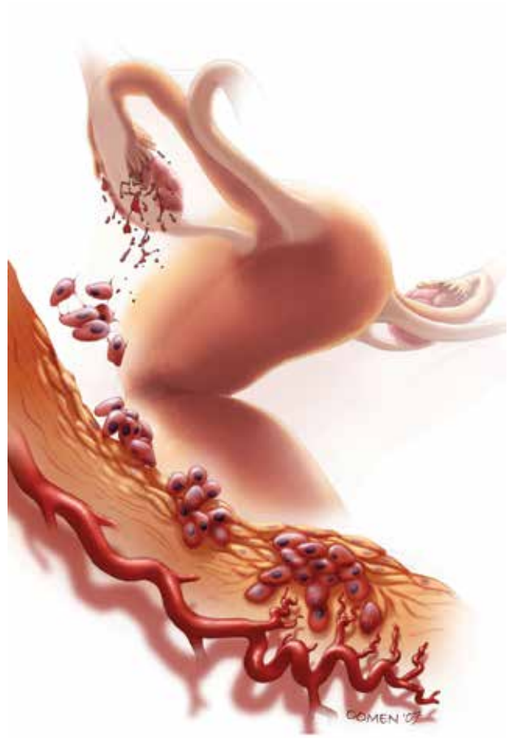
Professor Foster and his team recently undertook a clinical study in which they examined the levels of four factors called neurotrophins in the blood of women with endometriosis, compared with the levels in the blood of healthy control patients. Of the four factors examined, one, called bone derived neurotrophic factor (BDNF), proved to be highly expressed in endometriotic patients, compared with healthy controls. In addition, levels of this factor were a sufficiently sensitive and specific metric to predict whether the endometriosis was stage 1 or 2. Most excitingly, levels of BDNF in patients who were receiving certain types of therapy, including oral contraceptives, or 3 months after surgical removal of lesions, were similar to levels in the control group, suggesting that BDNF is a treatment-sensitive biomarker for endometriosis. Previously, the team showed that BDNF is expressed in the endometrium and a recent proteomics study showed that it is more highly expressed in the endometrium of women with endometriosis. While the exact role of BDNF in the pathophysiology of endometriosis is incompletely understood it appears to be a promising biomarker candidate for diagnostic purposes.

Future work and acknowledgements

The team wish to further examine the relationship between levels of BDNF in the blood and disease stage and severity and will investigate potential correlations between BDNF levels and levels of pain, quality of life scores, and lesion appearance.

Previous work from the team has compared levels of BDNF in the blood of patients with surgically confirmed endometriosis with levels in healthy controls. However, to fully understand the diagnostic potential of BDNF, the team aim to carry out a comparison to determine if levels of BDNF can be used to distinguish between patients with surgically confirmed endometriosis and patients with chronic pelvic pain but no symptoms of endometriosis at surgery. If BDNF levels could be used to measure such a distinction, then patients with chronic pelvic pain but no endometriosis might not have to undergo an invasive laparoscopic procedure and similarly, may not have to undergo ineffectual treatment with endometriosis medications, saving money and preventing patient morbidity.

The team plan to examine other potential markers of endometriosis (CA-125, VEGF, and IL6) to see if the sensitivity and specificity of a panel of markers is superior to the predictive value of a single biomarker, such as BDNF alone. Finally, the team have proposed a novel way to treat endometriosis, partially based around their findings regarding BDNF. Patients with endometriosis are often faced with a dilemma, whereby they have to choose between hormonal therapies that suppress their symptoms and forgoing such treatments in order to attempt to get pregnant. For patients in the latter category, the team hypothesise that treatment with melatonin represents a viable alternative that could help with the symptoms of endometriosis while facilitating pregnancy. In a small clinical trial melatonin was shown to reduce BDNF levels and improve pain scores in patients with endometriosis. The team will examine the effects of melatonin on endometriosis and fertility in a mouse model of endometriosis to determine if this approach is viable for patients attempting to get pregnant.



Professor Foster sees biomarkers for endometriosis and smartphone technology working in tandem in the future to help some endometriosis patients to monitor their disease severity and modulate their therapy appropriately. ‘Patients could use an app on their phone that alerts them to an impending flare up and the need to start medicating to prevent symptoms. This would be a relatively novel way of managing endometriosis and may be suitable for many (but not all) sufferers.’

Professor Foster is keen to acknowledge that this work has been a team effort. ‘Our work is the combined effort from a group of very talented and dedicated investigators,’ he tells *Scientia*, ‘others involved in this work are Dr Nick Leyland and Dr Sanjay Agarwal, my student Dr Jocelyn Wessels along with the tireless contributions of Annette Bullen. Other students involved in seeing this project through are Maria Haikalis, Allegra Drum and Eli Crapper, each of whom has carried out assays that have allowed us to show that brain-derived neurotrophic factor is potentially a very suitable candidate marker for the diagnosis of endometriosis. Their experimental results have allowed us to build a really strong research program in endometriosis that has enabled us to make important contributions in defining suitable clinical markers of endometriosis. This is very much a team effort that could not have had a modicum of its current success without the efforts of everyone mentioned above.’

Meet the researcher

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Professor Warren Foster is a Professor in the Reproductive Biology Division in the Department of Obstetrics and Gynaecology at McMaster University. Professor Foster has expertise in toxicology and reproductive biology. His research program is focused on the molecular and biochemical mechanisms underlying the female reproductive system and related disease processes. In particular, he is interested in endometriosis and the key strands of his research in this regard involve discovering novel biomarkers for endometriosis and developing novel therapeutic strategies for endometriosis. He has received funding support for his research activities from the Canadian Institutes of Health Research. Professor Foster is a recipient of a Canadian Institutes of Health Research/Ontario Women's Health Council mid-career award, an Ontario Women's Health Council career award and several student/postdoctoral fellow supervision awards.

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THE IMPORTANCE OF COPPER IN THE HUMAN CELL

Dr Sharon Ruthstein researches the biological pathways involving metal ions, especially copper, by using Electron Paramagnetic Resonance (EPR) Spectroscopy. Here, she discusses the importance of copper in the human body and the implications for future diagnostic and therapeutic care.

What is your educational and research background and what triggered your interest in research of the biological pathways involving metal ions?

When I did my PhD at the Weizmann Institute in Israel, under the supervision of Prof. Daniella Goldfarb, I acquired my expertise in EPR Spectroscopy. After graduation, I started my postdoctoral work at the University of Pittsburgh (PA) under the supervision of Prof. Sunil Saxena who is interested in the coordination of copper ions in various proteins. Here, I noticed that many proteins contain metal ions. I thought it would be interesting to use these paramagnetic metal ions in EPR spectroscopy instead of the commonly used site-directed spin labelling method, to resolve biological questions.

What is the importance of metals, such as copper, in living organisms?

Metal ions are involved in a variety of important biological and chemical processes at in the cell, including oxygen transport, biosynthesis, electron transfer and drug metabolism. Copper, similar to iron, is involved in electron transfer reactions and oxygen metabolism. Copper is also crucial for the development of the central nervous system.

Metal ions are a double-edged sword. Metal ions act as cofactors for catalysis in various enzymes but are also highly toxic. So, precise monitoring of the concentration of each metal is critical to the cell.

If we look at copper within living organisms, what do you think is the most important step in the copper cycle that we need to understand?

To date, it is known that there are three different pathways involved in the copper cycle in human cells: to cytochrome C, to SOD1, and to the Golgi apparatus. I believe that the most important pathway is the transfer mechanism of copper to the copper transporter "Atp7b" of the Golgi apparatus via the copper transporter "Ctr1" and through the copper chaperone "Atox1". This cycle controls the copper concentration within the cell and is therefore of tremendous importance for the copper regulation. Mutations in the copper transporter Atp7b were found to be responsible for Menkes and Wilson disease.

Why does the localisation of copper within the cell need to be controlled so carefully?

High concentrations of copper can be deleterious, leading to oxidative damage of proteins, lipids and nucleic acids. The coordination of copper has been linked to promoting peptide aggregation which results in forming amyloid plaques characteristic for Alzheimer's, Parkinson's and prion disease. On the other hand, copper deficiency is just as dangerous. This results in metabolic abnormalities due to a decreased function of copper dependent proteins.

Disruption of the copper homeostasis can eventually lead to neurological disorders. So, it is of vital importance to understand every step of the human copper cycle. With this knowledge, we can build a fundamental understanding of possible disruptive elements to the copper homeostasis.

At the moment, you are working on different research projects in your lab such as developing highly sensitive detection tools. When we look at clinical practice, what do you hope to contribute?

We are developing copper biomarkers for hypoxic tissues, tissues that lack oxygen supply. For this, we use detailed knowledge of the pathway and reaction mechanism or a compound. We hope that our compound will either replace the currently used biologically active tracer molecule in PET/SPECT imaging (FDG), or complement it so our knowledge of tumour size and type will improve leading to superior diagnostic examination in cancer patients.

I have also read a research proposal in which you hope to introduce a new treatment for ALS. What do you hope to achieve with this research to alleviate the burden of ALS?

I strongly believe that our lack of knowledge of the origin and causes of amyloidgenetic diseases like Alzheimer's disease, Parkinson's disease and ALS is the reason for lacking good therapeutic solutions. In my opinion, because these diseases are all related to high copper concentrations, shedding light on the copper cycle will result in more effective therapeutic approaches. Possible new therapeutic options could be the use of competitive metal ions as well as gene therapy, a growing field nowadays.

EXPLORING THE BIOLOGICAL PATHWAYS OF COPPER IN THE HUMAN CELL

Metal ions are involved in many important processes in the human cell. However, little is known about the regulation of these metal ions at cellular level. Dr Sharon Ruthstein of the University of Bar-Ilan researches the biological pathways of metal ions, especially copper, by using Electron Paramagnetic Resonance (EPR) Spectroscopy.

Metal ions

Metals are cofactors in many biological and chemical reactions in human cells, including oxygen transport and drug metabolism. More than 30% of all proteins in the cell use one or more metals to perform their specific functions and over 40% of all enzymes contain metals. On the other hand, metal ions can be highly toxic when free in biological fluids. So, a regulatory system is in place in the human body to manage the concentrations and types of metal ions in and outside the cell. Despite these regulatory systems, diseases such as Menkes, Wilson's, Alzheimer's, Parkinson's and Prion which originate from metal binding to proteins, still emerge.

The importance of copper

Copper is an important metal ion in the human body. It is involved in electron transfer reactions and oxygen metabolism. Copper is also crucial for the development of the central nervous system. High concentrations of copper can be dangerous, because it leads to oxidative damage of proteins, lipids and nucleic acids. Copper deficiency is just as dangerous resulting in abnormalities in the metabolism.

Copper accumulates in the human body through diet. After a change in its atomic structure, copper is delivered to the cell by the copper transporter called "Ctr1". After arrival in the cell, specific copper chaperones are responsible for delivering the copper to specific cellular pathways. Dr Sharon Ruthstein's lab focusses on the main copper transporter "Ctr1", on the "Atox1" copper chaperone and the "Atp7A and Atp7B" copper transporters. Mutations in "Atp7A" and "Atp7B" are found to be the leading cause for Menkes and Wilson's disease.

The "Atox1" copper chaperone and the "Ctr1, Atp7A and B" copper transporters are proteins that directly interact in a copper stimulated-manner.

EPR Spectroscopy in copper research

The main tool in the research of metal ions used in Dr Ruthstein's lab is continuous wave (CW) and pulsed electron paramagnetic resonance (EPR) spectroscopy. Over the last decade, EPR spectroscopy has emerged as an important method for resolving structure-function relationships in proteins. The power of this tool lies in the sensitivity to both atomic level changes and nanoscale fluctuations. EPR can characterize certain properties of the protein's functional state as well as information of its dynamics. EPR can also probe small fluctuations in the protein's structure. And, more importantly, measurements can be conducted in conditions that closely mimic the natural environment of the protein. EPR does not require crystallization and it is not limited to the protein's size. EPR is the perfect tool to target complex biological systems.

Dr Ruthstein uses EPR spectroscopy together with site-directed spin labelling techniques to explore structural changes that occur in the methionine motif when it binds with copper. Methionine motifs are methionine-rich metal binding segments present in many human proteins. These proteins are involved in the transportation of copper to other cellular pathways and in the protection of copper from oxidation. These methionine motifs bind copper as well as silver, another metal ion. Proteins that can bind different metal ions in similar way should be able to identify between them so they can shuttle them to their own pathway in the cell. Dr Ruthstein showed that the methionine segments undergo different structural changes while binding copper instead of silver.

She also looked at the copper transporter "Ctr1" as mentioned earlier. With this study she found out that the methionine motif, the methionine-rich metal binding segments in proteins, changed it's conformation when approaching the Ctr1 transporter. She discovered that these changes where dependent of which metal ion it was bind to, copper or silver.

The future in EPR spectroscopy

Studying the interactions between proteins involved in the metal cycle is often difficult. For instance, a protein undergoes only minor changes in its conformation when binding to metal ions. These minor changes can only be studied with biophysical tools that have a very high resolution. High resolution tools are an important step forward in the research of the functions of proteins at molecular level. It remains however difficult to study these proteins in a living environment, called in-vivo. In living cells, the proteins interact with other proteins, nucleic acids and co-factors. It is very difficult to replicate the cellular environment outside the living cell, called in-vitro.

So, there is a tremendous need to develop a highly sensitive, high resolution biophysical tool that will be able to provide structural and molecular information on the interactions between proteins which are involved metal ion transfer in and outside the living cell.

There are two major challenges in resolving the biological pathways that involve metal transfer in the cell. Firstly, it is necessary to follow the biological pathway in its natural environment. Secondly, the biophysical tool need to be highly sensitive to even minor changes in the conformation that the proteins undergo as a consequence of metal binding or protein-protein interaction during the biological pathway. In recent years, the biophysics community has been struggling with the development of these high-sensitive tools for in-cell detection. Dr Ruthstein is working on a new EPR and labelling methodology. This tool is based on time-resolved EPR measurements of photo-induced radical pairs (RP) and will be able to resolve the biological pathways with higher sensitivity and resolution, both in-vitro as in-cell.

Implications for clinical practice

CT and MRI imaging have long been the standard diagnostic tools in cancer research. However, CT and MRI are only limited effective in determining the metabolic and functional information of certain tissue. For this purpose, PET and SPECT are much more powerful techniques. There techniques are able to image an increasing variety of physiological phenomena because of the possibility to select a radio-tracer that specifically targets a particular mechanism. Because the incidence of cancer grows, there is continuous demand for the development of new radio-tracers for early cancer detection and chemotherapy targets.

A feature of all solid tumours is hypoxia which is the deprivation of adequate oxygen supply. The association between a tumour and low oxygen levels makes it a high priority target for cancer therapy. Dr Ruthstein is developing a highly sensitive copper-tracer for PET measurements using the gained knowledge on the copper transfer mechanisms which can be used in diagnostic procedures in cancer. She consults with renowned oncologists in Israel and the USA to further improve its diagnostic and therapeutic options in cancer.

Meet the researcher

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Dr Sharon Ruthstein received her Ph.D. in chemistry, with honors, from the Weizmann Institute of Science in Israel. She has been recognized by the Wolf foundation with the Krill prize for excellent young scientists in 2015. She received the Auto Schwartz prize from the Weizmann Institute in 2007. Currently, she is a senior lecturer and researcher at the department of chemistry of the Bar-Ilan University where she is particularly interested in the copper homeostasis and the structure and function of proteins.

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אוניברסיטת בר-אילן
Bar-Ilan University

A NEW FRONTIER FOR BIPOLAR DISORDER

Dr Charles L. Bowden, an expert in the field of bipolar disorder research, has defined the symptoms and biology of bipolar disorders. Here he discusses the obstacles patients and physicians face when assessing and managing bipolar disorder and how developments made through his research are helping to improve the long-term out look for patients with bipolar disorders.

To begin, please describe your research background and how you decided to focus your efforts on bipolar disorder specifically?

When I was a medical student at Baylor, one of my professors, Roger Guillemin, who was later awarded the Nobel Prize for his research on hypophyseal portal transport impacting anterior pituitary systems, drove home the likelihood that brain functions in discrete areas were under agonist/antagonist controls which could reveal part of the pathophysiology of brain-based disorders. In addition, during my psychiatric residency at Columbia, I had an assignment at a state hospital where thousands of chronically psychotic patients were indefinitely hospitalized. With the introduction of the first generation of antipsychotic and antidepressant drugs during this same period of time, most were soon able to re-join life in communities. As part of an obligatory two-year physician draft in the U.S. during the Vietnamese war, I was director of a narcotic addiction treatment unit. This led to a grant for addiction treatment utilizing methadone. All of these experiences heightened my resolve to focus my medical career on research involving brain-based disorders. My research deals with developing better assessment tools and improving diagnostic criteria, in particular, distinguishing depression as it appears in major depression from that in bipolar disorders.

It is clear from your work that there is a need in the healthcare community to provide a better means to aid mental health researchers and providers in assessing the trajectories of bipolar disorder. Can you describe the major conundrum in the field of psychiatry/ psychology that led you to address this issue?

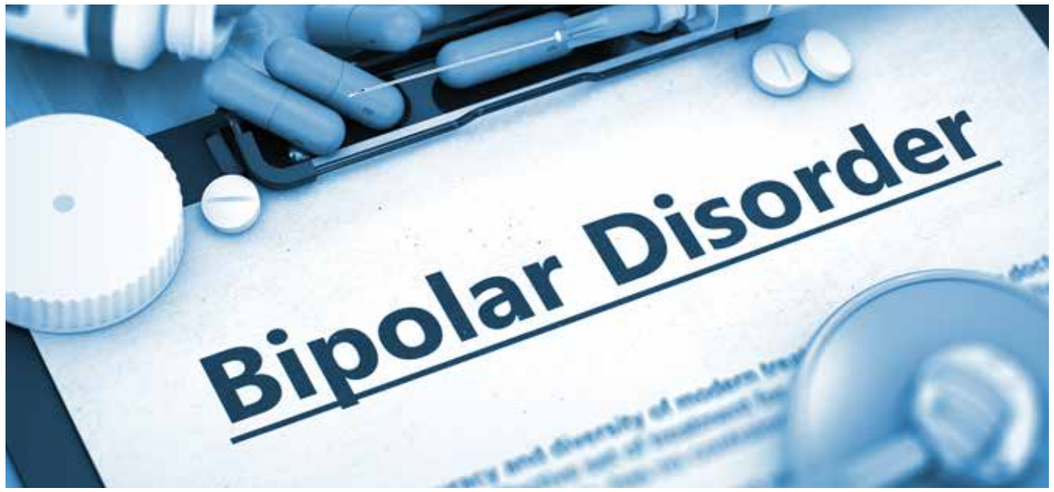
Bipolar disorder is differentiated from major depression because of it is associated with an earlier age of onset, much more affective lability and more anxiety. Bipolar mood disorders are associated with high rates of bipolar disorder in relatives. Unfortunately, official diagnostic guidelines have generally lagged in incorporating some of these data. An additional factor that is uncommon in most other major disease groups is the manic or hypomanic characteristics of bipolar that are not readily recognised as indicative of low-grade manic symptoms. In fact, the increased energy, complex thoughtfulness and productivity have long been recognised as characteristics of bipolar disorders in any age group, but are unlikely to be the only set of symptoms. In addition, some circumstantial factors are important in recognising bipolar symptoms, including circumstances that interfere with getting to sleep, e.g., noise, light, pain, which may be sufficient to move a person from a euthymic mood state to one of significant mania.

You recently published data describing a new model (MOAT) for assessing bipolar disorder that improves upon the limitations of the traditional Kaplan–Meier survival techniques method of assessment. Can you describe the approach you developed and why it was necessary to develop this for bipolar disorder?

The current survival analyses are limited to determining whether an event (e.g., relapse, death, discontinuation for intolerable side, or meeting a single severity score for either or both mania and depression) is the only item assessed for the main aim. No assessment of the quality of time prior to the target event occurs. A survival analysis does not assess the experiences of the many people who never have the target event. Based on studies that led to the regulatory approval of lamotrigine for mood stabilisation, a MOAT analysis was designed. MOAT addresses several misconceptions about what survival analyses can achieve. A survival analysis provides no information about either mood stability or subthreshold symptom intensity. Similarly, a patient might experience an early relapse followed by maintenance of a stable, episode free state, or subsyndromal status, neither of which is captured by survival methods.

The diagnosis and treatment of bipolar disorder seem to be evolving as technology advances. What do you see are the biggest changes that patients will see over the next 10 years?

By incorporating the new tools established in our research unit, patients with bipolar will have more access to the evidence on which diagnoses are arrived at and have a better understanding of actions that can forestall worse outcomes. A change we will not likely see is a major expansion of medications that benefit some core manifestations of bipolar. Much of this is consequent to FDA policies, which tend to push pharmaceutical companies to continue use of the same study designs that have been in use for over 50 years.



NEW TOOLS FOR BIPOLAR DISORDER MANAGEMENT

Bipolar disorder is a complex disease and self-management is an important component of the treatment. Dr Charles L. Bowden of the University of Texas Health Science Center at San Antonio has developed better assessment tools for bipolar disorder and for improving diagnostic criteria.

A Better Tool for Bipolar Disorder Assessment

Bipolar disorder is a manic-depressive illness that involves shifts in mood, energy and activity levels. The symptoms are severe and can damage relationships and lead to the inability to do a job or perform in school and even suicide. Historically, the diagnosis of bipolar disorder is limited to defining the manic or depressive states. Currently, studies have established that several symptom clusters characterise the illness, including readily identifiable anxiety, irritability and psychotic symptoms, each of which may be associated with either traditional depressive or manic symptoms. Dr Bowden's research aims to develop better assessment tools for improving the diagnostic criteria and for tracking patient outcome. In particular, his team aims to distinguish depression as it appears in major depression from that in bipolar disorders. Traditionally, these distinctions were done by utilising historically based criteria that are only weakly useful.

The current differentiators incorporate family history, illness course, biological systems that are disease specific and the recognition of specific symptoms and treatment response.

Dr Bowden believes that better tools are necessary to assess patients with bipolar disorder and this led to the development of the Multi-state Outcome Analysis of Treatments (MOAT) methodology. Chronic diseases are typically studied by statistical techniques that assess time to an event, such as the Kaplan-Meier (KM) survival analysis. However, the variables involved in studies analysed by survival analyses assume that certain events, including relapse or termination for an adverse effect, will happen to all of the patients, and this is not the case for bipolar disorder. MOAT is different because it accounts for the fact that such events happen to only a subset of patients, but it also can accommodate the shifting status of tolerability of patients with one treatment but not another. These factors make MOAT a more useful tool for evaluating bipolar disorder.

MOAT vs. Survival Analyses

The KM survival analysis is one of most commonly used survival analyses. The principle behind it is a 'time to event' model, which means the endpoint of an event is used to predict a time when certain positive events or negative events will occur. This method has limitations when estimating the outcomes of numerous variables, which is the case for bipolar disorder. If one uses the KM analysis for bipolar disorder, the variables being tested do not accurately display what is going on with the patient. Dr Bowden's group aimed to improve upon this limitation and they developed a tool that incorporates data on both the efficacy and safety applied to the time spent in primary clinical states of bipolar disorders to direct the analysis of illness trajectories and treatment selection.

A KM survival analysis tells the patient or the psychiatrist that more patients dropped out with treatment X than with Y, but this does not help with how to treat the patient. Furthermore, the outcome criteria in the KM survival analyses can be biased by designs that are pre-selected based on the positive or negative responses that are requirements to enter a treatment trial. MOAT is designed to avoid such distortions and also provides meaningful data that can be used by the patient and the physician.

Putting MOAT to the Test

Very recently, Dr Bowden and his colleagues published the results of a study that

describes the development of MOAT and it's application in assessing patients who were part of 2 FDA studies regarding the treatment of bipolar disorder. These FDA studies are what led to the approval of the drug lamotrigine for the treatment of bipolar disorder. There were a total of 578 patients and 224 were assigned to the drug lamotrigine, 165 to the drug lithium and 189 to placebo. The MOAT analysis was used to partition the total survival time of the patients into clinically distinct periods of bipolar disorder that were operationally defined by cutpoints on rating scales. These distinct periods were remission, subsyndromal and syndromal mania, mixed states or depression. Previously, it was shown that both drugs were associated with a longer time in the study compared with the placebo, but the MOAT analysis takes these findings to the next level and demonstrates that both drugs increased the time remitted compared with placebo. In addition, patients on all 3 treatments spent a large amount of time in subsyndromal depression. Lithium reduced the time with manic symptoms but lamotrigine did not. Lithium also had a worse tolerability compared with placebo. In summary, lamotrigine showed a limited therapeutic benefit but no harm, and lithium was both beneficial and harmful.

These results are important because they provide specific details that can be of direct use to patients as well as psychiatrists. In addition, regulatory staff can use the MOAT analytic data to create more useful label descriptions of the benefits as well as limitations of the benefits of drugs. Dr Bowden and his group hopes to stimulate other groups conducting longitudinal studies to use MOAT. In addition to bipolar disorder he believes that MOAT can be used for other generally common disorders with waxing and waning fundamental symptomatology and/or functional status. These could be other psychiatric disorders, such as post-traumatic stress disorder and major depressive disorder, or general medical and surgical conditions, such as neuropathies, hypothyroidism, diabetes, restless leg syndrome and hypercholesterolemia.

The Next Frontier in the Treatment and Management of Bipolar disorder

For patients with bipolar disorder, self-management is an important component of the treatment, but this is very complex and comes with difficulties, including misunderstanding of the condition and a lack of self-awareness. The emergence of mobile health and computational tools, such as applications used on a mobile phone, are allowing patients to engage in self-management by giving them timely access to information. Recently, Dr Bowden's group developed a patient-centred software system that is downloadable on smart phones to assist patients in the self-management aspect of bipolar disorder. They developed a computational tool which determines the precise state of a bipolar patient by tracking interacting symptoms. These include 8 symptoms that were selected independently from the Bipolar Inventory of Symptoms Scale by a panel of experts as essential to characterising the current state of a bipolar patient. After establishing the patient's state and trajectory, advice, specific to the patient's condition, is generated to help manage the course of the disease.

A small, 12-week field trial of the tool was conducted and completed with 20 patients. Next, Dr Bowden and his group are conducting a 12-month, randomised open comparison of the downloadable mood-tracking tool. Currently, the study has enrolled 100 subjects from three academic medical centres. The hope is that through the use of personalised self-management programs, patients will be more aware of their emotions and their environment, which could help to prevent relapse while focusing on avoidance based goals and actions.

Meet the researcher

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Dr Charles L. Bowden received his MD from Baylor College of Medicine in Houston, TX and completed his psychiatry residency at the New York State Psychiatric Institute and Columbia-Presbyterian Hospital in New York, NY. He received the 2001 Klerman Senior Investigator Award, the 2006 Mind of America Scientific Research Award from the National Alliance for the Mentally Ill and the 2008 NARSAD Falcone prize. In addition, he received the 2016 Andrew C. Leon Distinguished Career Award of the International Society for CNS Clinical Trials and Methodology. He has authored more than 400 publications and has been principal investigator for over 90 studies principally funded by NIMH.

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A BRIEF HISTORY OF PHARMACEUTICAL SCIENCE

Although medicines have been used by human beings since ancient and even prehistoric times, modern pharmaceutical science is said to have emerged in the early 1800s, with the isolation of many pharmaceutical compounds from plants. One of the first of these was morphine, which was first isolated from opium in around 1804. A second big leap for pharmaceutical science happened in 1820, when quinine, a common treatment for malaria, was first extracted from cinchona bark and colchicine, used to treat gout, was isolated from autumn crocus.

The ability to isolate and purify these medicinal compounds represented a huge leap forward for medical science for a number of reasons. Firstly, this meant that accurate doses of the drugs could now be administered – something which had previously been impossible due to the fact that plants contain varying amounts of the active drug. Secondly, toxic effects due to other naturally occurring substances in the plant could now be eliminated. Finally, gaining knowledge about the chemical structure of the active compounds has allowed for the synthesis of structurally related compounds and thus the development of valuable new drugs.

In the 1800s, much pharmaceutical science revolved around pain relief. Ether was first administered as an anesthetic for patients undergoing for surgery in 1942, and this was followed by the introduction of chloroform in 1847 by the Scottish physician Sir James Young Simpson. Due to their patients being unconscious, surgeons could now pay careful attention to the prevention of tissue damage, and could carry out much longer, complex surgical procedures safely.

However, following surgery, it was still quite common for patients' wounds to become infected and many died as a result. Then in 1865, the British surgeon and scientist Joseph Lister initiated a new era of antiseptic surgery. This not only involved the introduction of gloves and other sterile practices, but also the use of phenol as an anti-septic agent. However, much earlier than this, an even more significant innovation to combat infectious disease was developed with the introduction of the smallpox vaccine. This began as early as the 1790s, when the English surgeon Edward Jenner noticed that milkmaids who had previously been infected with the relatively benign cowpox virus were immune to the much deadlier smallpox virus. This led to the development of an immunisation procedure, which used crude material obtained from the cowpox lesions. Then in 1885, a vaccination for another deadly viral infection – rabies – was developed by the well-known French microbiologist Louis Pasteur.

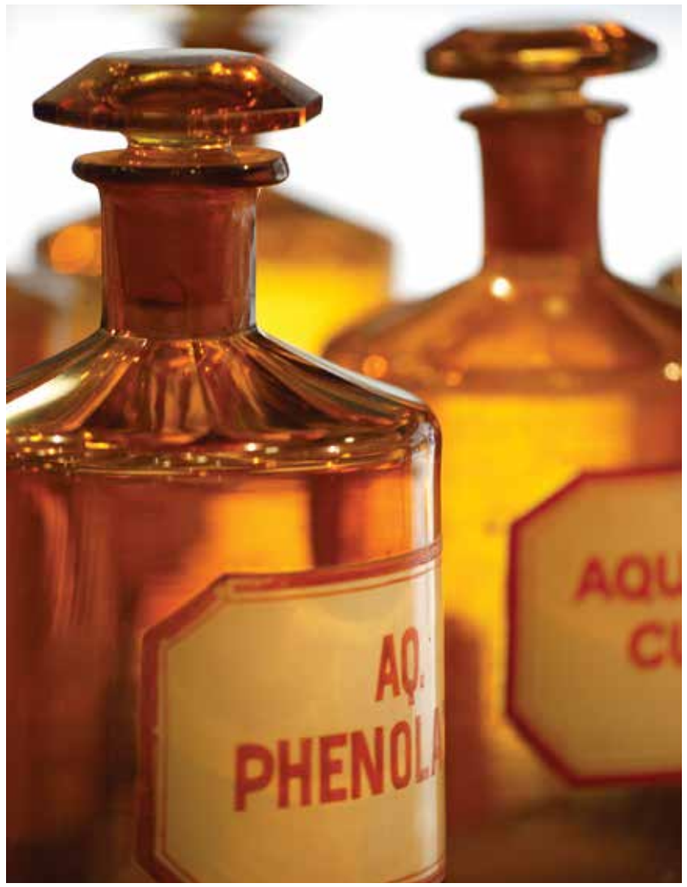
Working alongside vaccinations in the fight against infectious disease are antibiotics. The first chemical compound that was found to have anti-biotic properties is penicillin, in a serendipitous discovery made by Alexander Fleming in 1928, who noticed the inhibition of bacterial growth in a petri-dish that had become accidentally contaminated with blue-green mould. Certain types of penicillin are still widely prescribed to this day; however, many strains of bacteria have unfortunately developed resistance following the extensive use of these drugs.

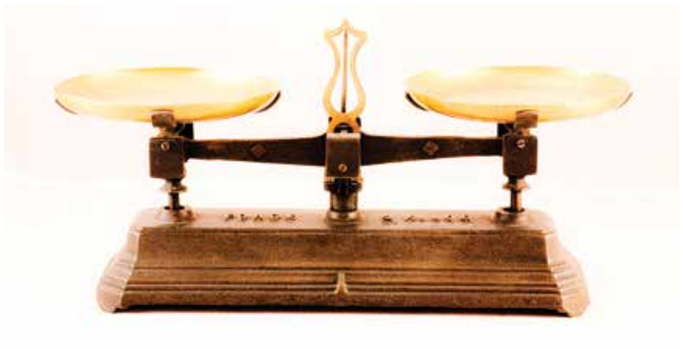
The development of both vaccines and antibiotics has led to a massive decline in the number of deaths that occur as a result of infection. This has led to life extension, thus unveiling a new breed of deadly diseases including cardiovascular disease, cancer and stroke – the three leading causes of death today. Advances in modern pharmaceutical science has led to huge progress in decreasing mortality caused by these diseases in even the past few decades alone.

Nowadays, pharmaceuticals are produced through the activities of a complex collection of public and private organisations, that work together towards the development and manufacture of drugs. Scientists at many publicly funded institutions such as universities carry out basic research in subjects from chemistry and physiology to microbiology and pharmacology, to name just a few. The results from these studies help to identify potential new targets for drug discovery, such as an enzyme, a drug receptor or a biological transport process. Once a target has been identified, pharmaceutical companies carry out much of the remaining work involved in the discovery, development, production and marketing of new drugs.

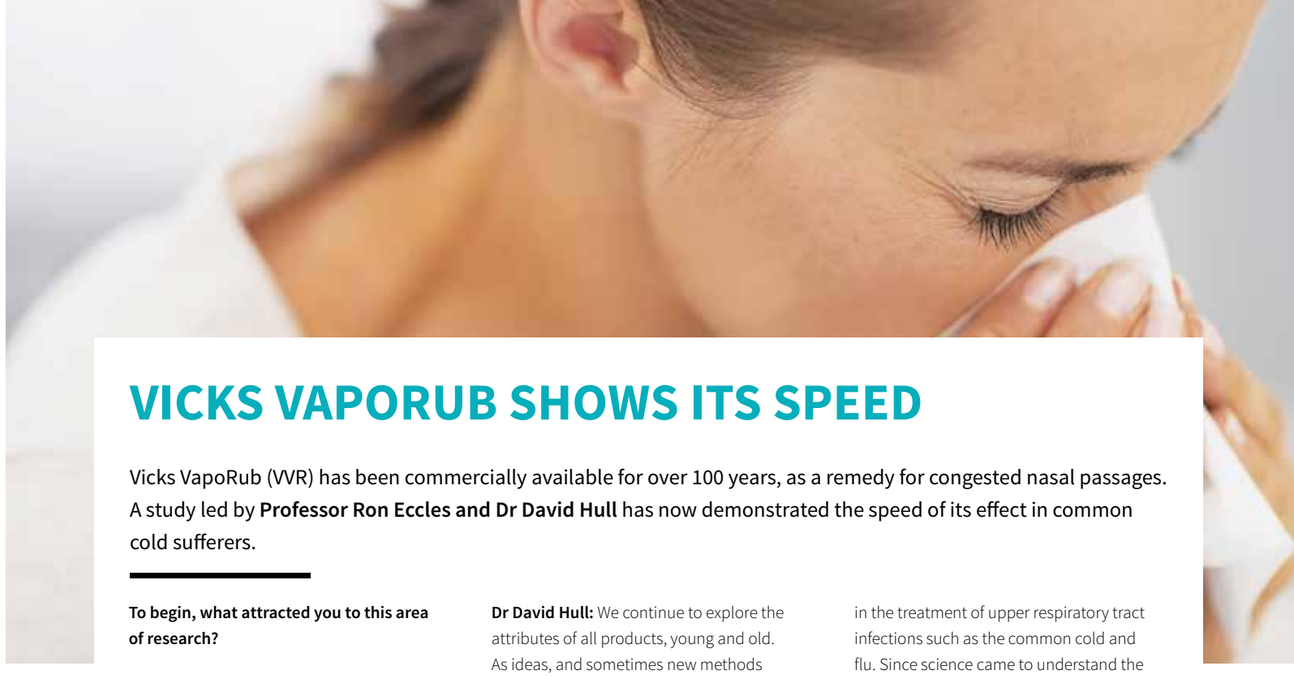
In this section of the edition we celebrate the collaboration between the public and private sectors in the development of pharmaceutical treatments. First of all, we expose the inner workings of a clinical trial, in a study designed to investigate the speed of nasal cooling and decongestion of Vicks Vaporub in common cold sufferers. This investigation was the result of a collaboration between researchers at the Common Cold and Nasal Research Centre at Cardiff University, Procter and Gamble and Teva Pharmaceuticals.

Next, we introduce quite a different type of clinical trial. This particular study, carried out by two Japanese scientists, Dr Osamu Hataji and Dr Esteban C. Gabazza, investigates the efficacy of a drug that has the potential to improve the treatment of chronic obstructive pulmonary disease (COPD). In this study, the researchers evaluate the effect of that indacaterol has on daily physical activity in patients with this common ailment that affects current and past smokers.





Finally, we highlight the activities of the Keesler Medical Center – one of the largest medical facilities in the United States Air Force. Located in Biloxi, Mississippi, this centre provides outstanding and affordable health care to approximately 7,500 active duty members in addition to 20,000 other enrolees. As the centre is also a hub of clinical research, in this article we describe one of their latest clinical trials, which investigates the effects of the drug tocilizumab on reducing major adverse events following heart attack.



VICKS VAPORUB SHOWS ITS SPEED

Vicks VapoRub (VVR) has been commercially available for over 100 years, as a remedy for congested nasal passages. A study led by **Professor Ron Eccles and Dr David Hull** has now demonstrated the speed of its effect in common cold sufferers.

To begin, what attracted you to this area of research?

Professor Ron Eccles: During my modular zoology undergraduate course at Liverpool University, I chose to do a module in pharmacology. I found the investigation of how drugs work in humans an amazing and exciting area of study and decided to switch my undergraduate studies to pharmacology. I was lucky enough to be offered a PhD scholarship after completing my undergraduate studies, and I chose to study the pharmacology of the nose, as I could see this was a very under-researched area. I fell in love with the nose during my research as there were always more questions than answers in this area, and I felt that I could contribute to knowledge in this area. When I got my first job at Cardiff University, I continued with nasal research and found a problem that did not seem to have an answer. How does menthol work on the nose to provide relief from nasal congestion? Menthol was an ingredient in a lot of Vicks products such as their 'Vaporub', inhalers and nasal sprays, but when I looked at the chemical formula for menthol it did not have any of the properties of a drug that would work as a nasal decongestant by constricting nasal blood vessels. I wrote to the Richardson Vicks Company based near London, UK in 1976 expressing my interest in the mechanism of action of menthol, and after several years of discussion and meetings I got the first of many research grants to study this mechanism.

The formula for VVR was developed over 120 years ago and is one of the most well-established household cold remedies. Why perform this study now?

Dr David Hull: We continue to explore the attributes of all products, young and old. As ideas, and sometimes new methods emerge, we strive to bring this to bear by gathering an improved understanding of their effects. We knew that VapoRub was fast-acting (just open the jar and you can feel an effect), but we had not tried to quantify that before and we did not know if the feeling extended from just cooling to an actual sense of decongestion. In conversations with our internal experts, we concluded that this would be worthwhile testing so that we could obtain a clear appreciation of 'just how fast' VapoRub is.

Furthermore, we know that there isn't a great deal of awareness of the clinical efficacy studies of VapoRub amongst healthcare professionals so we recognised this as an opportunity to build on our clinical knowledge.

Do you anticipate the results of this study strengthening sales of VVR? Or is there a strong separation between R&D and marketing at Procter and Gamble?

Dr David Hull: We in R&D always hope that our work will support the commercial success of our products. We believe that effective communication to healthcare professionals and patients is crucial to this, so we would expect these results to be valuable to our colleagues in Marketing as they communicate with both constituencies.

Besides potentially improved sales of VVR, what is the wider impact of this research?

Dr David Hull: I believe that the results of this study affirm the place of aromatic oils in the treatment of upper respiratory tract infections such as the common cold and flu. Since science came to understand the receptor biochemistry of these substances, the exploration of their effects has been more easily explained. Also as we now have a receptor-based pharmacology for aromatic oils to work with, we can better plan experiments such as this one in the expectation of an interesting and valuable result.

Did they get it exactly right with the original formula or do you think it can be improved? What else does the future hold for this product?

Dr David Hull: Well, I must begin by complimenting Mr Richardson, who developed such a clinically valuable therapy presumably by trial and error back in the 1890s. Is it beyond improvement? I doubt it. We continue to explore possibilities for this product and as we find new information we will be bringing it to the public domain. As most of our work is proprietary, I think my answer has to be 'watch this space'!

Are there plans for future studies into VVR efficacy?

Dr David Hull: We continue to listen to the patients who use our products and explore new science as it emerges to guide our work. We will therefore continue to look at Vaporub to see if we can understand better the benefits that it provides. If new ideas emerge, then we will test them.

MEASURING HOW RAPIDLY VICKS VAPORUB EXERTS ITS PHARMACOLOGY

Researchers at the Common Cold and Nasal Research Centre at Cardiff University, in collaboration with PGT Healthcare, a joint venture between the Procter and Gamble Company and Teva Pharmaceuticals, have investigated the speed of nasal cooling and decongestion of Vicks Vaporub in common cold sufferers.

The history of Vicks Vaporub

Vicks VapoRub (VVR), was developed in North Carolina in the 1890s. It quickly established itself as one of the best-selling cold remedies at the time and has remained a household staple ever since. With half a million Facebook 'likes', availability in over 60 countries, worldwide production in excess one million gallons per year and sales totalling over one billion units in the last five years alone, the popularity of Vicks appears unshakeable.

The original formulation was created by Greensboro pharmacist, Lunsford Richardson who purportedly borrowed the name 'Vick' from his brother in-law, Dr Joshua Vick, because it sounded snappier than Richardson. Though he concocted various other remedies such as Vick's Chill Tonic, Vick's Little Laxative Pills and Vick's Tar Heel Sarsaparilla, nothing quite compared to the success and popularity enjoyed by VapoRub.

Richardson's death in 1919 resulted rather ironically, from the Spanish flu pandemic which had propelled VapoRub sales from $900,000 to $2.9 million in a single year. The company he founded, Richardson-Vicks, was bought by Procter and Gamble (P&G) in 1985.

'After decades of research we now know that menthol acts on TRPM8 sensory nerve receptors in the nose to cause a cool clear sensation of nasal airflow and this is the basis of its action in providing relief from nasal decongestion.'

R&D at P&G

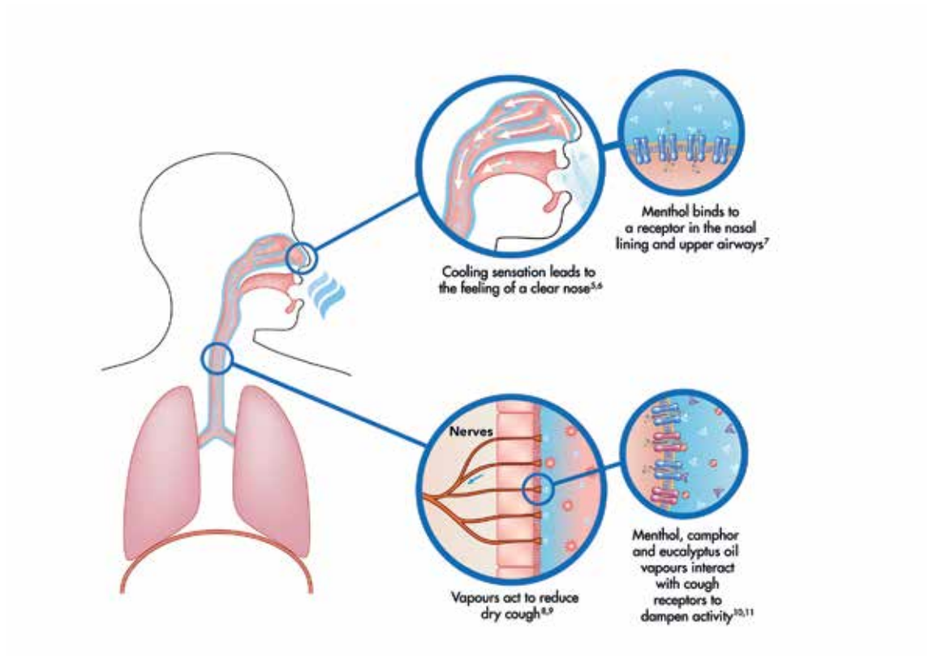
P&G have conducted various studies into the efficacy of VVR for cold and flu relief, demonstrating that it reduces cough frequency relieves nasal congestion and lasts up to eight hours. Their most recent study, published in the Open Journal of Respiratory

Diseases in collaboration with researchers at the Common Cold and Nasal Research Centre at Cardiff University, focussed on the speed of action of VVR, compared to a petrolatum control using a group of 50 common-cold sufferers. Cold and flu sufferers report that one of the main desires for any medication is a feeling of rapid relief from nasal congestion, as this symptom interferes with day to day activities, can prevent sleep and is generally uncomfortable.

The results showed that VVR produces nasal cooling and congestion relief significantly more rapidly than the control, though after a longer period of time, the petrolatum control was also reported to produce these effects. In the treatment group, VVR produced a sensation of nasal cooling around 23 seconds after application (around 99 seconds for the control), while nasal decongestion was achieved after around 62 seconds (126 seconds for the control). When asked about this placebo effect, David explains, 'In one sense, if we had not seen a placebo effect we would have doubted our results. All medical treatments and procedures are subject to this effect. The question is whether we can account for it satisfactorily. The difference observed provides confidence that the VapoRub mediated effect was clinically more significant.'

Blinding an odour

Their study was single-blind (blinded to the investigators only), due to the nature of the product. The distinctive odour of VVR is inextricably linked to its activity, so the patient will immediately know whether they are in the control or treatment group, once



their nose clips are removed following application. The nose clips were worn in order to standardise the start time of the inhalation process, though the investigators' nose clips remained on for the duration of the experiment. The participants were instructed to stop two clocks which were started when the nose clips were removed, the first one upon the sensation of nasal cooling and the other upon experiencing nasal decongestion. The expectation of these two events may have been a strong factor in the observed placebo effect in the control group.

The researchers also suggest that the placebo effect could have been exaggerated within the control group resulting from the way the nose clips were used in the study. The clips were applied to patients before entering the study area and remained on during the application of the product. This inevitably means that the patients are mouth-breathing, and there is no airflow through the nose during this period. Upon removal of the clips, the control group may have experienced the sensation of nasal cooling, not as an effect of the petrolatum, but resulting solely from the removal of nose clips after wearing them for 3–5 minutes. 'There is no getting away from the placebo effect,' says David, 'the trick is to design the study such that the performance of the active product can be reliably assessed. This study design achieved that.'

The pharmacology of Vicks

There is a scientific explanation behind the activity of VVR, largely based on the interaction between the vaporised active ingredients and receptors found within the sinuses. VVR is paraffin-based and contains levomenthol, eucalyptus oil, turpentine oil and camphor as its active ingredients. These are inhaled when applied topically to the chest and throat, and are evaporated by body temperature, or if added to hot water.

The menthol and eucalyptus oil in the formulation interact with a receptor located within sensory neurons in the nose which are also responsible for the detection of cold temperatures. This interaction causes the sensation of nasal cooling, feeling similar to breathing cold air through the nose. The nature of this interaction was not elucidated overnight. 'Studies on the D- and L-stereoisomers of menthol lead me to believe that menthol works on a very specific receptor in the nose, as the L-isomer gave relief from nasal decongestion but the D-isomer was inactive,' explains Ron, 'after decades of research, we now know that menthol acts on TRPM8 (also known as CMR1 – cold and menthol receptor 1) sensory nerve receptors in the nose to cause a cool clear sensation of nasal airflow, and this is the basis of its action in providing relief from nasal congestion.'

Other uses and the future of Vicks

A difficult-to-test concept for a future study would be to investigate the efficacy and speed of action of VVR specifically on coughing, a symptom which VVR is widely accepted to provide relief from. This is harder to investigate however, due to the sporadic nature of coughing. This would arguably be even more subject to the placebo effect, as coughing is often a conscious decision. A study performed on children with upper respiratory tract infections did show that an application of VapoRub caused reduced nocturnal coughing, nasal congestion and sleep difficulty, based on surveys completed by their parents. This symptomatic relief also allowed the parents to get a better night's sleep themselves.

The results of the current study may help to reinforce to healthcare professionals that Vicks VapoRub should not be grouped into the long list of over-the-counter cough and cold remedies with dubious efficacy, as it actually has rapid and measurable effects. When asked what the future holds for Vicks, David says, 'watch this space'!

Meet the researchers

Professor Ron Eccles
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Dr David Hull
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Ron studied pharmacology at Liverpool University before specialising in the pharmacology of the nose during his PhD studies. He continued his research in this area at Cardiff University, and began working with Richardson Vicks in 1976, studying the pharmacological activity of menthol.

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David is a molecular virologist who originally studied the role of viral genetics in the assembly of viral particles. His PhD studies at Queen's University in Belfast were focused on the measles virus, which he then continued to study at Rockefeller University in New York. He later researched the influenza virus at Northwestern University, Chicago, before joining Procter and Gamble to investigate the pathophysiology of upper respiratory tract infections and the efficacy of non-prescription remedies.

After 30 years' experience in science, he has recently gone 'back to school' in his spare time, achieving a 1st class BA (Hons) in Philosophy and Religious Studies from the Open University in 2011 and an MA in Philosophy in 2015.

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AN IMPROVED QUALITY OF LIFE FOR COPD PATIENTS

Dr Osamu Hataji is a physician who diagnoses and treats various respiratory and lung diseases. **Dr Esteban C. Gabazza** is a professor with expertise in inflammation and the immune system. Here, they discuss COPD treatment and how they are working together to assess physical activity in COPD patients as a method of treatment.

Dr Hataji, as a clinician, could you tell us about what it was that first drew your interest to medical research?

During my routine clinical practice, I encounter many challenges. This involves not only assessment and diagnosis of a patient's symptoms and conditions, but also trying to identify other potential underlying causes. Much of my passion and attention has been focused on the work of pursuing and ultimately capturing such "truth".

Your trial investigates a drug that has the potential to improve the treatment of chronic obstructive pulmonary disease (COPD). Could you please explain the major causes and prevalence of this condition, and tell us about the impact that it has upon a patient's quality of life?

The main cause of COPD is smoking, and the mortality of COPD is highly associated with a patient's level of physical activity. COPD patients have difficulty breathing and, as a result, become less and less physically active. This lack of physical activity then causes even more difficulty in breathing. It's a vicious cycle that, unfortunately, many patients often fall into. And this is precisely why bronchodilators are so important for improving a patient's breathing and overall health. However, as we make use of bronchodilators, it is important for us to closely monitor patients in order to evaluate improvements in their level of physical activity.

Dr Gabazza – you have research interests throughout the field of immunology. What brought COPD to your attention and how does that fit within the larger scope of your research.

After I took up my current position as Head of the Immunology Department I built up a large research group consisting of basic scientists and clinicians. Initially we focused on asthma, and we have gradually moved towards COPD as the needs are greater in this area of research. I have always been aware that COPD is a major clinical problem, and at the time I got involved in this research very little was known about the underlying cellular and molecular mechanisms, and so we developed a new animal model to look at the nature of the inflammatory response in COPD. The promise is that our research will lead to new treatments. Contrary to asthma, therapy for COPD is less effective and there are no drugs that repress the chronic inflammation and lower progression and mortality of COPD.

In this study you evaluate the effect of indacaterol on daily physical activity in patients with COPD. Could you please explain the rationale behind routine physical activity as a measure of the efficacy of indacaterol treatment?

The mortality of COPD is highly associated with a patient's level of physical activity. Bronchodilators significantly improve pulmonary function, physical endurance and subjective symptoms. However, there has not been any report as to whether bronchodilators can improve patients' physical activity level. That is why I decided to conduct research to find out whether indicaterol can improve a patient's level of physical activity.

How does indacterol act to alleviate the symptoms of COPD? How does this improve upon traditional bronchodilators?

Indacaterol works as a broncodilator. In the past, anticholinergic agents were used as the first line treatment for COPD; failing that, beta-agonists were considered the next best choice. As it turns out, for treating COPD, however, beta-agonists are considered to be generally ineffective. That said, ultra-long-acting beta(2)-adrenoceptor agonists, such as indicaterol, have actually been found to be as good, if not better, when compared with anticholinergic agents.

Dr Gabazza – Your laboratories' research theme is 'The development of new strategies to prevent and cure chronic inflammatory diseases', in addition to COPD, what are your other promising areas of research?

Our focus is to clarify the molecular and cellular mechanisms of inflammatory responses in environmentally (including cigarette smoking and alcohol drinking) and intrinsically stimulated organs. We are particularly interested in the functional relationship between inflammation and the coagulation-fibrinolysis system.
We try to figure out the behaviour and function of dendritic cells under the control of coagulation factors, and to reveal mechanisms of immune activation. In order to obtain outcomes that can be utilized to resolve pathogenicity, and to prevent human diseases, we adopt the latest technologies and devote ourselves to our research.

TREATING COPD BY IMPROVING PHYSICAL ACTIVITY

COPD (chronic obstructive pulmonary disease) is a common ailment that affects current and past smokers and currently there is no cure. Treatments for COPD improve some of the symptoms, but more need to be done to improve the quality of life for these patients. Dr Osamu Hataji, director of the Respiratory Center, Matsusaka Municipal Hospital, and Dr Esteban C. Gabazza of the Mie University Faculty and Graduate School of Medicine are working to develop ways to monitor the physical activity in patients with COPD before and after the use of drug treatments.

COPD and the Vicious Cycle

COPD stands for chronic obstructive pulmonary disease. A person with COPD has a hard time breathing, and this gets worse as time goes on. COPD causes excess mucus to be produced due to coughing, and also leads to wheezing, shortness of breath and tightness of the chest. The leading cause of COPD is cigarette smoking. Most people with COPD have smoked in the past or are current smokers. The prevalence of COPD is high. It affects more than 10% of the population worldwide and is expected to be the 3rd leading cause of death by 2020.

COPD develops slowly. The science behind COPD involves a chronic inflammatory process. This means that there is an excess of immune cells in the airway of the lungs that cause damage to the tissue. This damage limits the normal airflow in the lungs. One of the main complaints from COPD patients is a shortness of breath and this hinders physical activity, which worsens the quality of life for these patients. To improve the symptoms of COPD, patients are told to increase their physical activity. The problem is patients with severe COPD are often unable to perform basic activities like walking, cooking, or taking care of themselves. This lack of physical activity increases the difficulty in breathing, which is a vicious cycle that, unfortunately, many patients often fall into.

Treatment for COPD patients

Currently, there is no cure for COPD, and therefore, a major research goal is to find treatments that reduce symptoms and improve the quality of life for the patients. Current treatments include bronchodilators, rehabilitation and home oxygen therapy. Inhaled long-acting bronchodilators are very

effective at improving symptoms, exercise capacity and the quality of life, as well as for preventing exacerbations. Although, it is unknown whether bronchodilators improve a patient's physical activity level. This is where the work of Drs Hataji and Gabazza comes into play. Together, with their colleagues, they conducted a study using the drug indicaterol to determine if the drug improved a patient's level of physical activity.

Indacaterol is a type of drug called a ß2-agonist and it functions as an ultra-long-acting bronchodilator that has an established clinical efficacy and safety profile. It was approved for clinical use by the European Medicines Agency in 2009 and by the US Food and Drug Administration in 2011. This drug only needs to be administered once a day, and for patients with moderate to severe COPD, it significantly improves the symptoms, pulmonary function test results, and quality of life. In addition, recent studies have demonstrated that indacaterol might also improve exercise endurance in COPD patients. The effect of this drug on daily physical activity in these patients is not well understood.

Assessing physical activity as part of COPD treatment

Movement of the body that is produced by skeletal muscle contraction and leads to energy expenditure is referred to as physical activity. When evaluating the therapeutic response in patients with COPD, the level of physical activity during routine daily life is now recognised as an important outcome parameter. Patients with limited physical activity have more hospitalisations because of exacerbations, and signs of physical activity predict mortality in patients with COPD. In the past, researchers have validated the use of accelerometers (devices that detect motion) to obtain an objective measurement of physical activity in patients with the disease. In the study conducted by Drs Hataji and Gabazza, they assessed the effect of indacaterol on the daily physical

COPD patients have difficulty breathing and, as a result, become less and less physically active. This lack of physical activity then causes even more difficulty in breathing. It's a vicious cycle.

activity of patients with COPD using a uniaxial accelerometer.

Their study included 23 patients with COPD who were part of the outpatient department at the Matsusaka Municipal Hospital in Japan. All of the patients were former smokers and, at the time of the study, were not using any pharmacotherapies to help them stop smoking. All of the patients had stable disease with no exacerbations prior to or during the study. The study design was set up so that 14 of the patients received tiotropium, which is a long-acting, 24-hour, anticholinergic bronchodilator that is used in the management of COPD together with indacatoerol and the remaining 9 patients received indacatoerol alone. The study design is considered as an open-label clinical trial with no randomisation, no placebo group and no blinding.

In order to record and assess the physical activity changes in the patients, a Lifecorder, which is a uniaxial accelerometer, was attached to their belts for 4 weeks before they started receiving the indacaterol therapy and then for another period of 4 weeks while they were receiving treatment with the inhaled indacaterol. This was a way to compare the physical activity of the patients before and after treatment with indacaterol.

The Lifecorder measured the number of steps, the duration of moderate or higher degree exercise, the energy expenditure and the metabolic equivalent of task in all of the patients before and after treatment. In all of the patients, the number of steps, the length of moderate or more activity and the energy expenditure all were significantly increased after treatment compared with before treatment. In addition, the metabolic equivalent of task was also significantly improved after treatment with indacaterol compared with before the treatment, suggesting that this ß2-agonist improves routine daily activity in patients with COPD. Drs Hataji and Gabazza used a novel accelerometer that had been previously evaluated for sensitivity and efficacy. They suggest that the use of this novel accelerometer is effective for assessing the daily physical activity of COPD patients because it is easy to use, cost-effective and can record data in a real-time basis over a long period of time.

The Future of Physical Activity Assessment: The Smart Watch

According to the World Health Organization (WHO), it is recommended that within a week's time span all adults should participate at least 150 minutes of moderate-intensity aerobic activity, like walking, in order to maintain a healthy lifestyle. For patients with a medical condition like COPD, this is a difficult task. The 2013 Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy advised that all patients with COPD should participate in daily physical activity, but the level at which the activity it supposed to be conducted was not defined. Even with such recommendations, patients with COPD report less physical activity compared to patients with other diseases, such as rheumatoid arthritis and diabetes. The assessment of that physical activity is also part of the puzzle, as Drs Hataji and Gabazza have alluded to. Objective measures of assessing physical activity continue to be investigated through clinical evaluation and validation. Accelerometers, like the one used in the study described above, are electronic devices that are generally worn on the arm (like an armband) or waist. There is still limited evidence for the reliability, validity, and responsiveness of accelerometers in COPD patients. There are suggestions that they are sensitive to artefacts, observation bias and compliance issues. This suggests that there is room for improvement, as with any technology.

In a recent study by Drs Hataji and Gabazza, they evaluated the use of a Smart watch to evaluate physical activity in COPD. The validation of the Smart watch was done by correlating the results with data obtained from two types of accelerometers. For this study they monitored 10 male patients with stable COPD from the outpatient department of the Matsusaka Municipal Hospital in Japan. All of the patients were former smokers and were not undergoing any active therapy to stop smoking. The Smart watch, with a built in accelerometer, was compared to a uniaxial accelerometer, the Lifecorder and the triaxial accelerometer, the Actigraph. Each device measured the number of steps, the energy expenditure in calories, the time of exercise and the distance walked. The results demonstrated that the number of steps and the amount of calories measured by the Lifecorder and Actigraph was significantly correlated with all of the Smart watch activity parameters. These findings reveal that the capacity to monitor physical activity in COPD patients by wearable Smart watches is comparable to devices that are generally used in clinical practice, such as the accelerometers used in thus study.

As an added measure, Drs Hataji and Gabazza also confirmed the correlation between the pulmonary function tests and the physical activity variables measured using each device. They found that the physical activity variables measured on the Smart watch reflected the lung function in a manner similar to the currently used devices. In fact, some of the Smart watch variables reflected lung function even better than the Actigraph.

The use of Smart watches has become quite popular in the general population.

This recent study by Drs Hataji and Gabazza demonstrates how the potential applications of popular technologies can be investigated for use in in routine clinical practice. Significant correlation was found between the corresponding parameters of the Smart watch and traditionally-used devices, which suggests the potential applicability of Smart watches for monitoring physical activity in COPD patients. Future studies in a larger population will be required to confirm these findings.

What's next?

According to Dr Hataji, indicaterol is not the only bronchodilator he and his team are looking into. There are a few other bronchodilators that are currently being developed, and he would like to learn more about such treatment options. In addition, his group plans to conduct more studies to understand whether mortality decreases as the level of a patient's physical activity improves or increases.

Dr Gabazza states that his lab focuses on clarifying the molecular and cellular mechanisms of inflammatory responses in environmentally (including cigarette smoking and alcohol drinking) and intrinsically stimulated organs. He and his colleagues are particularly interested in the functional relationship between inflammation and the coagulation-fibrinolysis system. His team is searching for pharmacological control of inflammatory diseases and cancer and aims to apply these findings to clinical innovation that facilitates establishment of strategies for regulating the immune cells involved in disease progression.

Drs Hataji and Gabazza are clearly tackling a multitude of issues that surround COPD and this approach is sure to yield promising results for patients in the future.

Meet the researchers

Dr Osamu Hataji
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Dr Osamu Hataji diagnoses and treats various respiratory and lung diseases. He conducts a variety of clinical trials for respiratory diseases for promoting the development of new drugs. His team carries out multidisciplinary treatments in all areas, including respiratory medicine, respiratory surgery, radiology and rehabilitation.

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KEESLER MEDICAL CENTER'S DEDICATION TO EXCELLENCE

Located in Biloxi, Mississippi, Keesler Medical Center is one of the largest medical facilities in the United States Air Force. It provides outstanding and affordable health care to approximately 7,500 active duty members in addition to 20,000 other enrolees.

General highlights

Keesler Medical Center has 60 beds, of which 10 are in the Intensive Care Unit (ICU). This hospital serves active duty personnel, their families, retired service members, and Veteran Affairs (VA) centres. Facilities include seven recently renovated surgery suites, a newly built inpatient building, and a Radiation Oncology Center. The state of the art Family Birthing Center is another main feature of this centre.

There are about 60 services and education programs available. Furthermore, Keesler Medical Center is partnered with the University of Mississippi Medical Center, Gulfport Memorial Hospital, and the VA Gulf Coast Veterans Health Care System Medical Center. It is also affiliated with 35 institutions that are involved in training resident physicians and other medical professionals. The centre also focuses on medical readiness preparation in accordance to the Department of Defence guidelines.

Cardiology and other services

At the heart of a new drug trial, Keesler Medical Center hosts the busiest cardiology service in the Air Force. It performs more than 1,000 cardiac catheterizations yearly, of which a majority of the patients are stable and will likely benefit from a stent placement. Emergent cases account for the remaining number of catheterizations. Due to the high number of patients overall, the cardiology department can enroll sufficient numbers of participants in the tocilizumab study. This drug may benefit heart attack patients by decreasing their risk of developing severe and fatal complications that may occur afterward.

There are other specialties available at this Air Force hospital such as general surgery, gastroenterology, endocrinology, pulmonary and critical care, infectious disease, nephrology, orthopaedic surgery, obstetrics and gynaecology, rheumatology, and plenty of others as well.

Training future doctors

Keesler Medical Center provides physician training in internal medicine, which is a three-year program that educates medical residents in inpatient, ambulatory, and preventative medicine. Residents also train at other hospitals along the Gulf Coast to ensure a well-rounded and robust education. Furthermore, the program offers daily didactic lectures to develop and enhance critical reasoning skills in the residents and students. After graduation, the physicians are eligible for continued subspecialty training.

Other training programs include the general surgery residency, dental, and physician assistant as well. In addition to junior doctors, this facility educates medical students during their school rotations.

Research opportunities

Keesler Medical Center is a research site with established programs designed to promote clinical investigations associated with clinical medicine, graduate medical education, and Department of Defence. The hospital currently has 88 research protocols, including the tocilizumab drug trial. Medical investigators, including training clinicians, are actively publishing research, presenting at conferences, and seeking new and exciting collaborations.

Why Keesler?

In conclusion, Keesler Medical Center offers comprehensive medical care, top notch facilities, superior training and education, as well as cutting edge research.

A PROMISING TREATMENT FOR HEART ATTACK

Tocilizumab, a drug used in the treatment of Rheumatoid Arthritis, is currently being studied for its potentially beneficial effects on lowering complications after a heart attack. It does this by targeting a key player in the process.

The role of interleukin 6 in a heart attack

An acute myocardial infarction (MI), or heart attack, occurs when oxygen-rich blood cannot flow to blocked areas of the heart. The resultant process involves inflammation and injury to the cardiac tissue. A cytokine called interleukin 6 (IL-6) play a significant role in activating and recruiting many immune cells. The level of IL-6 is elevated in a heart attack and other stressful events. In fact, the amount of IL-6 remains persistently high even after a heart attack and has been linked heart damage during this time. Based on this information, a drug that blocks the IL-6 receptor is believed to produce significant improvement during and after myocardial infarction. The selected medication is tocilizumab.

The research trial

Keesler Medical Center in the Air Force Medical Service is investigating the effects of tocilizumab on reducing major adverse events after myocardial infarction. These serious complications include a repeat episode of MI, abnormal heart rhythms, swelling of the heart, fluid build-up around the heart, a tear in the coronary artery, or even death itself. This research study is designed to treat individuals who suffered a heart attack with tocilizumab within 24 hours of admission. Of course, the patients will still be treated with the routine medications and procedures. The investigators are hypothesizing that tocilizumab reduces the occurrence of these adverse events.

In addition to these potentially catastrophic effects, the trial analyses numerous secondary outcomes as well. It assesses whether the drug decreases the length of hospital stay, which is typically 24 to 96 hours. The study also evaluates readmission rates as this has implications regarding hospital costs and patient health. Furthermore, the investigation administers a thorough phone questionnaire 30 days after treatment, in which it determines the subject's symptoms and quality of life. Since tocilizumab has known side effects such as infection, reactivation of tuberculosis, and gastrointestinal perforation, the survey ascertains if the patient is experiencing these.

Another key component of the study involves testing for a biological marker known as C-reactive protein (CRP), which is directly related to IL-6 levels. CRP typically remains elevated for weeks after a heart attack. Therefore, measurement of CRP levels provides insight into the impact of tocilizumab on IL-6 and inflammation. Hence, CRP blood levels are obtained at four different times: 1) baseline (time of medication), 2) 24 hours after treatment, 3) 48 hours after treatment, and 4) 30 days after treatment.

Drug safety

The selected drug is considered safe for those who have undergone myocardial infarction. This is based on what investigators know about Rheumatoid Arthritis. Individuals with this disease have a higher risk of coronary heart disease and heart attack compared to the general population. In fact, they are at the same risk as those with diabetes mellitus. Subjects enrolled in this above study are carefully monitored in the hospital and pose no more risk than patients with Rheumatoid Arthritis, who self-inject this drug in the outpatient setting.

The participants

Due to the high number of patients with myocardial infarction and cardiac catheterization at this hospital, there are sufficient candidates for the study. Statistical analysis suggests that the trial should have at least 125 participants to yield significant data.

Patient information used in the overall evaluation includes age, gender, race, and body mass index (BMI). The two groups under investigation are subjects receiving a placebo and those receiving a tocilizumab injection. As with most trials, there are distinct criteria that the participants fulfil before being enrolled. Specifically, the participants are above 18 years old and are diagnosed with heart attack through comprehensive testing. Conversely, some factors will exclude patients from participating. These are individuals with infections such tuberculosis, HIV, or Hepatitis B or C. Additionally, those who have allergies to the drug, or are pregnant or breastfeeding are excluded.

Goals of the trial

While designing the trial, investigators did not know the actual impact of tocilizumab, but they are hopeful that prompt drug therapy will result in early blockade of IL-6, which in turn helps injured heart cells heal faster. Thus, a more rapid recovery is followed by relief of symptoms, a shorter hospital stay, and more importantly – prevention of another heart attack or other life-threatening medical conditions.

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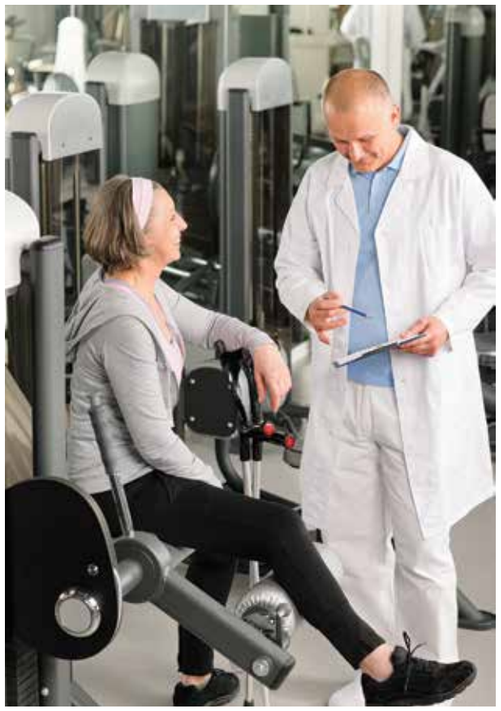
NON-PHARMACEUTICAL APPROACHES TO HEALTHCARE

One of the major advantages of non-pharmaceutical therapies, is the potential avoidance of the off-target effects that plague many common drugs and potentially dangerous drug interactions. In fact, non-pharmaceutical therapies such as physical exercise can have a wide array of positive off-target effects, from improved mental health to weight loss and increased cardiovascular health. In many cases, physicians think of disease as a puzzle to be solved pharmacologically and often overlook non-pharmaceutical therapies that can exploit the significant healing potential of our own bodies. Healthy diet, getting enough sleep and partaking in regular exercise can go a long way to preserving and enhancing health, but are not as simple a solution as taking a pill. The use of multiple drugs, especially in older patients, can lead to dangerous adverse reactions that can be life threatening or debilitating.

Harnessing non-pharmaceutical alternatives is the focus of this next section. In our first article, Dr Shun Takagi of Waseda University, Japan, investigates the potential for aerobic exercise to improve outcomes for patients who have had a heart attack. His results indicate that initiating aerobic exercise early during recovery can be beneficial. The second article in this section showcases the work of Dr Shin-Siung Jung, of the Everspring foundation in Taiwan, who has pioneered the use of sensory-motor integration training in children with attention-deficit/hyperactivity disorder, as a non-pharmaceutical alternative treatment. The training involves tasks that improve motor skills, such as using a basketball while in a prone position. However, the children who undergo this therapy demonstrate increased academic performance and reduced social withdrawal. Finally, we look at the work of Professor Philip Hazell, of Thomas Walker Hospital, New South Wales, Australia. Professor Hazell's work deals with investigating the effectiveness of developmental group psychotherapy in preventing self-injury amongst at-risk adolescents. Using a technique developed in the UK did not prove to be as effective in Australian teens, but Professor Hazell is hopeful that effective systems to reduce self-harm can be developed and implemented in the future.



ENHANCEMENT OF MUSCLE DEOXYGENATION IN ISCHEMIC HEART DISEASE

Dr Shun Takagi investigates exercise muscle physiology in patients who have recently suffered from heart attack. Furthermore, he studies the maximum oxygen uptake by skeletal muscle as a measure of the aerobic capacity in these patients.

Can you start by telling us about your research background and when you began your focus on patients with ischaemic heart disease?

When I was PhD student at Tokyo Medical University, I was interested in skeletal muscle oxygen dynamics during exercise. Some researchers in our laboratory managed the cardiac rehabilitation centre at Tokyo Medical University Hospital. Approximately half of our patients had ischemic heart disease, and ischemic heart disease is a primary disease of chronic heart failure. So, I began to examine whether the peripheral factors are impaired in early post ischemic heart disease patients.

It is well known that heart disease patients suffer from limited oxygen supply, caused by a suppression of the heart pump function. Recently, it has been established that not only central function (i.e. heart function), but also peripheral function (i.e. muscle blood flow and/or muscle metabolism) is impaired, especially in chronic heart disease patients. In contrast, in ischemic heart disease patients, it is generally believed that exercise tolerance is limited by heart function (i.e. ischemia of the heart), but it is unclear whether ischemic heart disease patients have impaired muscle function because of muscle deconditioning, which is considered to be one of the main reasons of impaired muscle function in chronic heart disease patients.

In your research, why did you choose to measure muscle oxygen dynamics and maximum oxygen uptake in these patients?

Increasing aerobic capacity is important for ischemic heart disease patients to improve their prognosis, and to accomplish this, an enhancement of muscle oxygen extraction may be needed. That's why we investigated muscle oxygen dynamics during exercise and their relationship to maximum oxygen uptake. We need to monitor the circulation and metabolism in exercising muscle during whole-body exercise and test the direct relationship between peripheral factors and peak aerobic capacity.

You used the Near Infrared Spatial Resolved Spectroscopy (NIRS) technique to measure certain variables in patients. How does NIRS work and what exactly does it measure?

The NIRS technique is a way to take measurements non-invasively during whole-body exercise, and it is widely used in exercise physiology, sports science and clinical science. To explain briefly, NIRS technique uses a wavelength in the range of 700–900nm to penetrate biological tissue. It reflects the oxygen dynamics in the arterioles, capillaries, and venules in exercising muscles. Muscle oxygen dynamics by near infrared spectroscopy reflects the balance between oxygen supply and utilization, and it estimates muscle oxygen extraction.

What effects of exercise did you observe regarding the maximum amount of oxygen in heart disease patients?

We observed that maximum oxygen uptake was heightened by aerobic training. Increased maximum oxygen uptake reduces cardiovascular-associated morbidity and mortality. Therefore, aerobic exercise training is an effective way for patients with heart disease to increase maximum oxygen uptake.

Based on your research, can you make recommendations for aerobic exercise in patients with ischaemic heart disease?

In our study, even though the amount of aerobic training was low (30 minutes of cycling at moderate intensity, 1 or 2 times a week for 12 weeks), aerobic training certainly improved maximum oxygen uptake safely in early after ischemic heart disease patients. It has also been reported that, in patients after onset of heart attack, early enrolment in aerobic exercise training is more effective for improving exercise capacity than later enrolment. From these findings, for early after ischemic heart disease patients, 2 times/week aerobic training (such as 30 minutes of cycling at moderate intensity) seems to be one of the best and safest options to improve aerobic capacity.

This appears to be a promising area of research. What further studies, if any, will you be working on?

We have some plans to look deeper into muscle oxygen dynamics and the relationship to peak aerobic capacity. In the subjects of our previous study, heart pump function, such as left ventricular ejection, was relatively preserved. There may be an interaction between muscle oxygen dynamics and preserved heart pump function. Also, to some extent, the preserved heart pump function may be related to improving peak aerobic capacity. Trainability for muscle oxygen dynamics may also be different, compared to ischemic heart disease patients who have reduced heart pump function.

IMPROVING POST HEART ATTACK OUTCOMES THROUGH AEROBIC EXERCISE

Dr Shun Takagi is conducting research trials to determine the effects of exercise on muscle oxygen uptake in patients who recently suffered from myocardial infarction (heart attack). The results point to the benefits of initiating aerobic exercise early after a heart attack.

In individuals battling long-term heart disease, the heart pump activity is profoundly affected. Hence, they exhibit a limited supply of oxygen. Also, their skeletal muscle function is impaired, which could be attributed to either reduced blood flow to the skeletal muscles or less metabolism taking place in the muscles. These patients also have muscle deconditioning, which may contribute to muscle physiology abnormalities. Likewise, those with ischemic heart disease also have altered muscle physiology as well as poor exercise tolerance resulting from suppressed heart function.

Dr Takagi hypothesises that a higher aerobic capacity results in better medical results in individuals with ischemic heart disease. Peak aerobic capacity is defined as the maximum amount of oxygen utilised by the muscles. He further speculates that increased muscle deoxygenation, or muscle usage of oxygen, will help achieve an improvement in aerobic capacity.

To aid the research studies, Dr Takagi and his team employ a non-invasive technique, referred to as Near Infrared Spatial Resolved Spectroscopy (NIRS). This method evaluates the balance between oxygen supply and use, as it assesses how much oxygen was removed from the blood. The NIRS technique measures the vastus lateralis muscle in the thigh for haemoglobin and myoglobin bound to oxygen, haemoglobin and myoglobin unbound to oxygen, the total concentration of these proteins, and oxygen levels in muscle. It utilises a wavelength in the range of 700 to 900 nanometres, which enters the tissue to specifically measure the oxygen levels attached to blood haemoglobin and myoglobin of the individual at rest in comparison to the levels during exercise. Haemoglobin and myoglobin are proteins that bind oxygen in the blood and muscle, respectively.

The effects of exercise in patients with myocardial infarction (heart attack)

In one of his research projects, Dr Takagi investigated the effects of aerobic exercise training and non-training in patients with recent myocardial infarction. The trial was conducted at the cardiac rehabilitation centre affiliated with Tokyo Medical University Hospital. In this study, the enrolled participants met several main criteria. First of all, the patients' myocardial ischemia had occurred less than six weeks prior. Also, the ages of the subjects fell between 40 and 75 years. Finally, the individuals demonstrated a capability of physical exercise. Moreover, they qualified by passing five cycling sessions. The patients were stable and received coronary artery stents. Additionally, they received their appropriate cardiac medications and were watched closely throughout the study. There were two groups of patients: 1) a training group which consisted of 10 patients and 2) a non-training group comprising six patients. Training consisted of moderate intensity cycling for 30 minutes, twice a week, for 12 weeks. The training sessions included warm ups and cool downs with a gradual increase in intensity. Before and after the 12-week period, the patients exercised up to the point of exhaustion. To obtain the required data, the NIRS technique was used to measure important variables.

The important findings demonstrate that the peak aerobic capacity was significantly elevated after 12 weeks in the training group, but remained unchanged in the non-training group. Additionally, the training patients showed enhanced muscle deoxygenation, which means more oxygen was used by the muscles. However, this was not observed in the non-training group. These results exhibit significant implications in terms of improving morbidity and fatality in heart disease. Therefore, it is paramount for patients with cardiac health issues to incorporate aerobic exercise in their course of recovery.

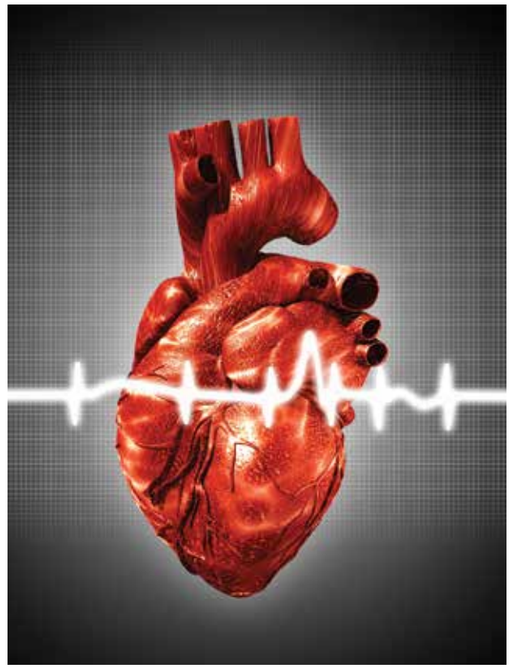
We observed that maximum oxygen uptake was heightened by aerobic training. Enhanced maximum oxygen uptake reduces cardiovascular-associated morbidity and mortality. Therefore, aerobic exercise training is an effective way for patients with heart disease to increase maximum oxygen uptake.

In another trial, the research team compared the muscle deoxygenation and peak aerobic capacity in two groups: 1) patients who recently suffered a heart attack and 2) normal healthy control subjects who were matched in age, height and weight to those in the first group. Specifically, the first group was made up of 16 patients who had experienced a heart attack 12 to 45 days prior to partaking in the study, while the control group consisted of 18 subjects. All of these subjects underwent ramp cycling until exhaustion. The findings revealed that muscle deoxygenation was blunted and markedly less in the heart attack group versus the healthy control group. These results are likely attributed to the lower use of oxygen, not decreased blood flow.

Collectively, these studies indicate that exercise improved the maximum amount of oxygen uptake in patients with cardiac ischemia. Furthermore, this enhanced muscle deoxygenation is likely correlated to increased peak aerobic capacity in these post heart attack individuals.

The effects of exercise in patients with angina pectoris

An additional study compared patients with angina pectoris and control patients, and evaluated the before and after effects of training in angina pectoris patients. The trial enrolled seven participants with angina pectoris and seven healthy subjects of similar age, height and weight as the patients in the first group. As for those with angina pectoris, they all had recently undergone coronary artery bypass grafting; specifically, their post-surgery timing ranged from 18 to 42 days. These enrolees trained with cycling activities for 10 to 20 half-hour sessions over 12 weeks. Before and after training, they performed a ramp bicycle exercise until exhaustion. Furthermore, the NIRS method was employed in this trial to take key measurements. The investigators observed that diminished muscle deoxygenation occurred in patients with angina pectoris compared with healthy matched controls. Furthermore, the investigators concluded that low volume aerobic exercise enhanced muscle deoxygenation and extraction in training angina pectoris.



Future research prospects

Dr Takagi and his colleagues have future plans to examine the relationship between muscle oxygen dynamics and the peak aerobic capacity in further depth. For example, based on the fact that the heart pump function is preserved in the patients previously studied, it would be beneficial to find its association with the oxygen dynamics.

Another aspect worthy of assessment is the occurrence of muscle deconditioning and when this happens in the heart attack timeline. Taking this a step further, he questions whether deoxygenation results from post heart attack deconditioning of muscles or if it develops prior to the heart attack.

Another important consideration for future projects is the expansion of the research trials described above. Enrolling a larger volume of subjects, especially women, will be pertinent to corroborate the findings yielded in those studies.

Applying these results in a healthcare setting

In one of Dr Takagi's main research studies, positive outcomes were observed in post myocardial infarction patients participating in cycling at moderate intensity for a duration of 30 minutes once or twice a week. Since this exercise regimen enhanced oxygen uptake by the muscles, it can be recommended as an option for patients who have recently experienced a heart attack. Moreover, it is better to begin physical exercise soon after a heart attack as opposed to later. While more frequent training with or without increased intensity can boost the aerobic capacity, this may not be feasible or convenient for the patient's schedule or health. In conclusion, there is promise for successful healthcare in patients who recently suffered a heart attack.

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Dr Shun Takagi is currently a researcher at the Faculty of Sport Sciences at Waseda University. He received a PhD from Tokyo Medical University. His focus is on exercise physiology with emphasis on muscle circulation and metabolism in relation to aerobic capacity. He was recognized with the Mishima Award and the Dietrich W. Lübbers Award for his excellent work. With funding from the Japan Society for the Promotion of Science, Dr Takagi is conducted research trials in skeletal muscle oxygen dynamics during exercise in patients with heart disease.

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BRINGING ADHD INTO FOCUS WITH NON-PHARMACEUTICAL TREATMENT

Dr Shin-Siung Jung, of the Everspring foundation in Taiwan, has pioneered the use of sensory-motor integration training in children with attention-deficit/hyperactivity disorder (ADHD), as a non-pharmaceutical alternative treatment. The training comprises a series of exercises involving prone extension postures, which cause the activation of the neocortical association pathway in the prefrontal lobes. The results to date indicate that this treatment approach is highly effective in reducing the symptoms of ADHD.

Attention-deficit/hyperactivity disorder

Attention-deficit/hyperactivity disorder (ADHD) is a psychiatric disorder, which is first diagnosed in childhood. It is characterised by an array of symptoms including problems with motor skills, hyperactivity, inattention, difficulties focusing on and completing tasks (particularly those which are perceived as not enjoyable, such as school work), impulsive behaviour, disruptive behaviour and difficulties in forming relationships. Not every sufferer will display all of these symptoms, and in order to be diagnosed, symptoms must be present for at least six months, must be significantly greater than what would be expected for children of a similar age and must be fully manifested before or at the age of 12 years. The social and academic consequences of ADHD can be significant, and the condition can also create a variety of problems for teachers and parents of affected children, who may demonstrate disruptive behaviours in the classroom and at home. These problems and their consequences can even manifest into adulthood. A study of adult men who had been diagnosed with ADHD as children found that they tended to have poorer outcomes in terms of social, economic and educational considerations, compared with adult men who were not previously diagnosed with ADHD.

The causes of ADHD are still not fully understood. It appears to be a combination of genetic and environmental factors. It is also believed that brain trauma can play a role, in certain cases, as can foetal exposure to alcohol or tobacco smoke during pregnancy.

Current treatments for ADHD

ADHD is typically treated using a combination of lifestyle changes, drug therapy and counselling, either alone or together. As the disorder presents itself differently in different patients, there isn't a 'one size fits all' solution to reducing the number and severity of its symptoms. Behavioural therapies form the basis of many counselling interventions and family therapy is also sometimes employed to train parents and guardians in the best way to deal and interact with a child experiencing ADHD. Commonly used drugs include stimulants such as Ritalin and Adderall to increase focus and attention span, and antidepressants such as Atomoxetine, a selective serotonin reuptake inhibitor. Drug therapy typically produces an improvement in symptoms, such as enabling increased academic performance, for the majority of treated patients in the short term, but whether it is effective in the long term is unclear. In addition, the use of stimulant drugs carries the risk of addiction or dependency. Finally, lifestyle changes, such as undertaking an exercise regimen, have been shown to improve symptoms in children with ADHD. Regular physical exercise, such as aerobic cardiovascular exercise has been shown to

improve focus and reduce some of the motor deficits present in ADHD. In addition, regular exercise as a therapy for ADHD has the added benefit of not producing adverse side-effects, while improving overall physical fitness, and so is a promising complementary therapy.

Sensory-motor integration training – rationale and application

Sensory-motor integration training is another promising therapeutic approach for children with ADHD. This training is based on the work of Dr Anna Jean Ayres of Southern California University, who posited that the integration and processing of sensory signals derived from the body (such as touch or movement) can greatly affect phenomena and abilities such as behaviour, emotional responses, learning and motor activity. Dr Ayres studied how the brain integrates visual, tactile, auditory, vestibular and kinaesthetic inputs in the brain stem. This information, which arrives in huge amounts, is processed in the brain stem and impulses are sent to the body and limbs and also to higher cortical centres for further processing. If this integration and processing is not efficient or is dysfunctional, this can result in sensory over-sensitivity or poor motor skills and can underlie learning difficulties and poor social abilities. Sensory integration theory is used as a means of assessing and designing treatments for people, who, within this frame of reference, are considered to have a dysfunction in sensory processing and/or sensory integration. An underlying principle of sensory integration training is that such sensory difficulties affect people with autism type disorders and some individuals with learning difficulties, and that sensory-motor integration training can be utilised to target and improve sensory integration and processing. Such techniques have been shown to address a variety of symptoms experienced by such individuals.

Dr Shin-Siung Jung tells Scientia how he first learnt how to apply the technique and how he brought it to Taiwan: ‘In 1983 I visited Dr Ayres’ clinic in Southern California. On coming back to Taiwan, I applied to Dr Mau Dien-Wun, Chief of Education of the Bureau of Taipei Metropolis, to establish a Sensory Integration Therapy room in a school, to be used for physical therapy by Physical Therapists from hospitals of the Taipei Metropolis. The Physical Therapists used the room for about one year with one training session per week on Saturday afternoons, without obvious or great effects.’

‘These accidental findings alerted me to the utility of this technique for ADHD’

Soon after, Dr Jung discovered that the technique could be extremely effective in treating children with ADHD. ‘Some parents of children with ADHD, who demonstrated the classic symptoms of hyperactivity and poor physical coordination, asked if their children could use the room.’ he explains. ‘In the room the children had discovered that they liked to play in a prone extension posture on scooter boards (a movable board with wheels) using a dodge-ball, during their midday resting interval. I was in charge of how to use the room and after discussion with the parents, I decided that under parental supervision, those children would be allowed to play dodge ball in the room, in the manner described above. The children played in the prone extension posture in two groups passing the ball between group members, from 8.00am to 8.40am before their school classes, from Monday through to Friday. Within two weeks of performing these exercises for 40 minutes daily before lectures, the supervising parents told me that these students became quieter, displayed more concentration in class, and wrote their home work with better handwriting. They also reported reduced levels of shouting and social withdrawal. Their teachers reported the same findings. These accidental findings alerted me to the utility of this technique for ADHD.’

Dr Jung’s results to date

Spurred by these promising, albeit preliminary results, Dr Jung and his team have applied the technique in larger, scientifically rigorous studies to more accurately determine the effectiveness of sensory-motor integration training in children with ADHD. In the latest study, a total of 94 grade school students with ADHD, who ranged from 7.5 to 10.1 years when they began treatment, were assessed before and after three months of sensory-motor integration training. The children undertook a variety of exercises while lying prone on a plywood scooter board mounted on four wheels. The exercises included pushing a ball against a wall, passing a ball to an adult in a sitting position and ‘walking’ on their hands. The duration of the exercises increased consistently during the treatment period, to build up the strength and ability of the children gradually. The children

were assessed using a battery of tests. The test results derived from observations and records made by their parents and teachers, which measured a variety of ADHD metrics such as academic performance, social interactions and motor function.

The results indicate that the exercise regimen significantly improved ADHD symptoms in the children who took part in the trial, with the exception of children who experienced temper tantrums as a result of cold or influenza during the testing period. Children who took part in more exercise sessions per week had greater test scores compared with those who undertook less exercise. Children demonstrated an improvement of 80% or over on nine items on the Teacher Rating Scale – a test assessed by the teachers of the children. These nine behaviours were ‘attack behaviour’, ‘clumsy movements’, ‘very clumsy at manual labour in school’, ‘difficulty with dictation or listening and writing’, ‘impulsive and irritable’, ‘creates chaos during class’, ‘easily distracted and poor concentration’, ‘reacts strongly to the touch of others’, and ‘frequently forgets to bring books or reports to classes’. When all the items in the Teacher Rating Scale were taken into account the mean total improvement was a whopping 72% ± 18%.

‘These students became quieter, displayed more concentration in lectures, and wrote their homework with better handwriting’

The team has also previously shown that the sensory-motor integration training has measurable effects on the brains of children with ADHD. Impressively, in a study they undertook in 2006, it was shown that the prefrontal lobes of students with ADHD demonstrated very limited activation when compared with healthy controls, but that after 3 months of sensory-motor integration training the scans of ADHD students were much closer in appearance to those of the healthy controls.

Subheading: Future outlook

The results generated by the research group to date have been extremely promising. However, Dr Jung would like to conduct a larger double blinded cross-over study, to more accurately assess the effectiveness of the sensory-motor integration training treatment approach in ADHD, and also to examine in more detail the mechanism underlying its effect on the symptoms of ADHD.

Dr Jung would also like to examine the possibility of using sensory-motor integration training to treat language and speech disorders in children. Language disorders occur when children have trouble understanding or using words in context. This can lead to difficulties in expressing or communicating ideas, problems with understanding or following instructions and difficulty in learning new concepts. The team have already observed some improvement in children with dysarthria who have been treated with sensory-motor integration training, and who haven’t received speech therapy. Dysarthria is typically characterised by motor deficits in the muscles that control speech, making audible and intelligible speech a challenge. Now, Dr Jung would like to assess if the technique can be used to treat children who have trouble understanding the meaning of words. ‘We have a new language disorder checklist, and are waiting to accumulate new data. We also plan to examine vestibular dysfunction causing language disorder.’ Dr Jung explains.

Hopefully in the near future, sensory-motor integration training will become a mainstream treatment option recommended by healthcare professionals. Presenting parents with the option to choose a healthy and drug-free treatment for their kids suffering with ADHD or language disorders, represents a huge leap forward for the future management of these conditions.

Meet the researcher

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Dr Shin-Siung Jung received his MD from Kaohsiung Medical University in Taiwan. He then went on to work at the National Taiwan University Hospital Neuropsychiatry Child Mental Health Center. Later he moved to the United States, where he took a post at Houston Texas Medical Center, and worked in the Department of Neurology, Baylor College of Medicine for three years. Upon returning to Taiwan he worked at St. Mary's Hospital of Lodung, as Chief physician of Neurology. He has also since held positions at National Taiwan University Hospital, and Taiwan Adventist Hospital.

In 1989, he founded the Everspring Foundation, which specialises in treating learning disabilities and ADHD in children through the use of sensory-motor integration. Dr Jung has published a total of 30 papers and 5 books.

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Dr Mau Dien-Wun, Chief of Education Bureau of Taipei Metropolis. Later being promoted to Chief Principal of Taipei University, and Chief Trustee of Everspring Foundation.

FUNDERS

In 1989, Dr Shin-Siung Jung donated 1000000 New Taiwan Dollar for the establishment of Everspring Foundation and selected Dr Mau Dien-Wun to be Chief Trustee for 25 years.
Later Dr Jung donated 500000 to 200000 each years, as general expenditure of Foundation.

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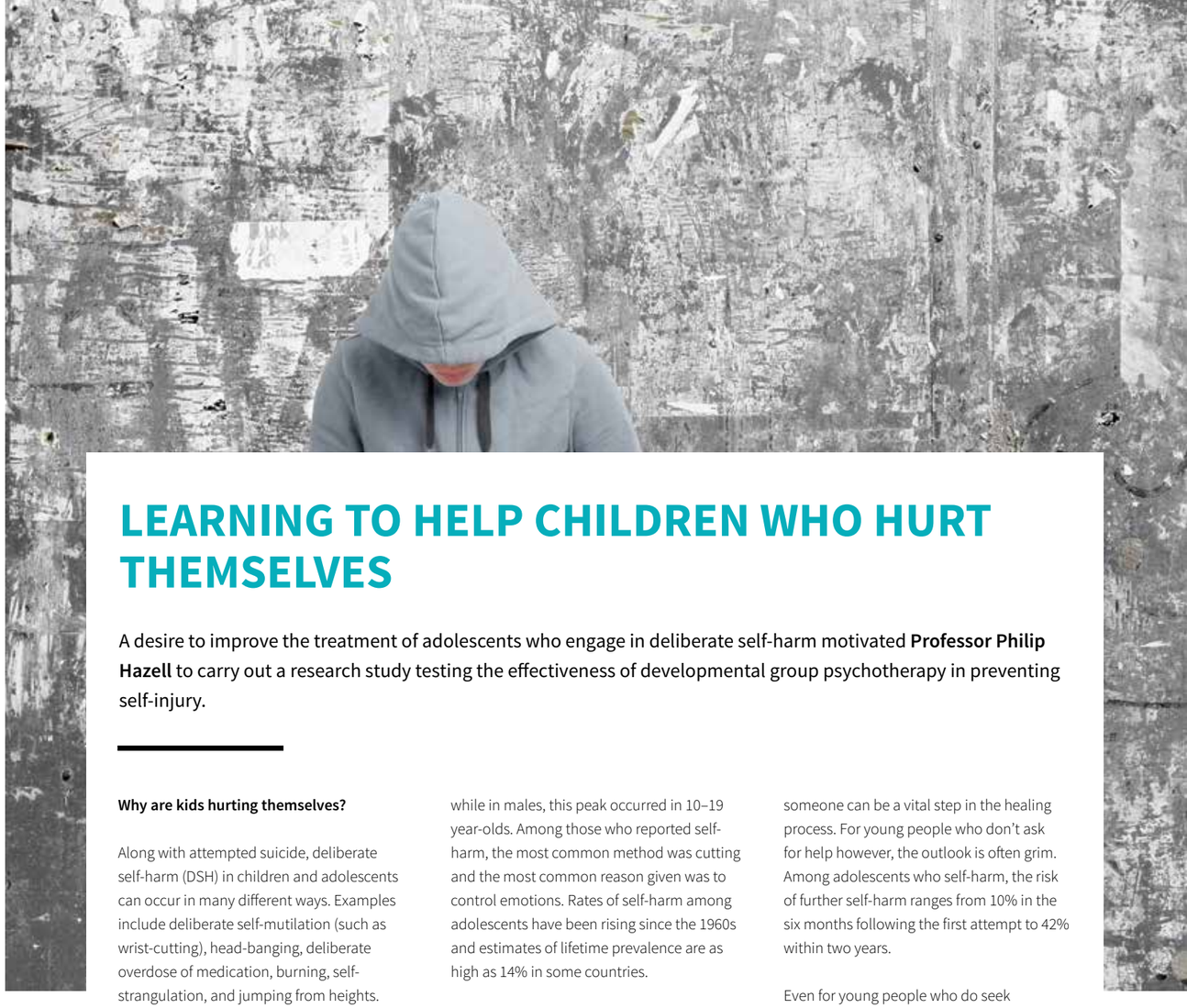

LEARNING TO HELP CHILDREN WHO HURT THEMSELVES

A desire to improve the treatment of adolescents who engage in deliberate self-harm motivated **Professor Philip Hazell** to carry out a research study testing the effectiveness of developmental group psychotherapy in preventing self-injury.

Why are kids hurting themselves?

Along with attempted suicide, deliberate self-harm (DSH) in children and adolescents can occur in many different ways. Examples include deliberate self-mutilation (such as wrist-cutting), head-banging, deliberate overdose of medication, burning, self-strangulation, and jumping from heights.

'About one in ten adolescent females and one in twenty adolescent males engage in self-harm', Professor Hazell tells Scientia. 'For many, it is a relatively benign behaviour which has minimal health consequences. For some, the problem becomes malignant, leading to disfigurement, frequent and problematic engagement with the medical system, and sometimes death. For these reasons, self-harm is an important public health issue.'

Community surveys looking at how often deliberate self-harm in children and adolescents actually occurs are just beginning to reveal the true magnitude of the problem. A 2010 community survey published in the Medical Journal of Australia showed that 1.1% of more than 12,000 Australians surveyed reported injuring themselves recently. For females, self-injury peaked in the 15–25 year-old age group, while in males, this peak occurred in 10–19 year-olds. Among those who reported self-harm, the most common method was cutting and the most common reason given was to control emotions. Rates of self-harm among adolescents have been rising since the 1960s and estimates of lifetime prevalence are as high as 14% in some countries.

Self-injurers generally report being more psychologically distressed than non-injurers. They are also more likely to have trouble managing their emotions, have poor problem-solving skills, and often have difficulty with relationships in general. In children and adolescents who harm themselves, most attempts occur in their own homes. Statistics also show that one in five self-harm attempts is alcohol-related, while one in eight occurs under the influence of drugs. A 2008 Australian study found that self-harmers were also more likely to have a history of childhood abuse and neglect.

Those same results show that less than half of all self-harmers actually seek professional help unless the self-harm attempt is particularly serious. However, most admitted to telling at least one person they knew about their self-harming. Whether this is a friend, family member or trusted teacher, being able to reach out and confide in someone can be a vital step in the healing process. For young people who don't ask for help however, the outlook is often grim. Among adolescents who self-harm, the risk of further self-harm ranges from 10% in the six months following the first attempt to 42% within two years.

Even for young people who do seek professional help however, finding the right kind of help is often a major challenge. Though general hospitals can deal with self-harmers on an emergency basis, providing them with help after they leave the hospital can be especially difficult.

Developmental group psychotherapy and self-harm

Treatment programs specifically aimed at helping self-harmers are still fairly rare. Most of the programs that are out there usually focus on related problems such as depression or substance abuse. This may explain why young people are often reluctant to seek treatment unless pressured to by medical doctors or family members. As for whether these different treatment options can actually help prevent future self-harm attempts, research studies tend to be pessimistic.

But what about a group therapy program specially designed for adolescent self-harmers? Could such a program provide a better treatment alternative to prevent future self-harm attempts? One new program developed by a clinical team from the University of Manchester and Greater Manchester West Mental Health Foundation Trust, seemed particularly promising. Known as Developmental Group Psychotherapy (DGP), this program is specifically intended for young people between the ages of 12 to 17 who have a history of self-harm.

Based on the principles of cognitive behavioural therapy, the DGP program also provides specialized training manual outlining different skills modules. These include social skills training, interpersonal psychotherapy and group therapy. By helping adolescents explore their relationships with family and friends, therapists encouraged them to become more pro-social. This means learning to overcome the isolation that can lead to self-harm. The first six program sessions focus on school and relationships, family problems, anger management, depression and self-harm, and worrying about the future. Following these first six sessions, adolescents then have the option of additional group sessions for up to twelve months.

According to a 2001 study looking at the benefits of DGP, adolescents receiving group therapy had significantly fewer self-harm attempts after 29 weeks compared to the routine care received by the control group. While these results appeared promising, they were still based on a fairly small sample (69 adolescents in total). Unfortunately, a later research study by the Manchester researchers using a much larger sample (366 adolescents evenly divided between treatment and control conditions) failed to show any real effectiveness in preventing further self-harm attempts.

Despite these conflicting results, developmental group psychotherapy remains one of the only real programs for helping adolescent self-harmers available. More research was definitely needed.

Bringing DGP to Australia

When asked about why he began researching adolescent self-harm, Professor Hazell replied 'When I return home from a session in the surf, my wife usually asks me if I caught any waves. Sometimes the answer is "no, but a few waves caught me". Self-harm is like a wave that caught me. The issue is unavoidable; it is in my face'. Along with being Conjoint Professor of Child and Adolescent Psychiatry at Sydney Medical School, Professor Hazell is also Director of Child and Adolescent Mental Health Services for the Sydney Local Health District and Director of the Rivendell Child, Adolescent and Family Mental Health Service in New South Wales. 'Self-harm, and concerns about risk of self-harm, are behind most of the after-hours calls I receive,' Professor Hazell explains, 'ever since my junior registrar days I have thought, "we can probably do this better"'. This motivated him to test the effectiveness of the Developmental Group Psychotherapy program in preventing self-harm in adolescents in an Australian setting, in a new research study in collaboration with a team of fellow researchers at the University of Newcastle and the University of Queensland. This new study, which has been published recently in the Journal of the American Academy of Child and Adolescent Psychiatry, looked at adolescents between the ages of twelve and sixteen who had least two self-harm episodes in the three months prior to the study. 138 adolescent self-harmers were referred by adolescent mental health agencies in three Australian cities. Of these, only 72 adolescents agreed to take part in the study.

'Self-harm, and concerns about risk of self-harm, are behind most of the after-hours calls I receive. Ever since my junior registrar days I have thought: "we can probably do this better"'

These participants were then randomly assigned to either the group therapy condition or the control condition. The 37 adolescents assigned to the control condition received routine care, including individual counselling, family therapy, and meetings with a case worker. For the group therapy condition, 35 adolescents received the same kind of programming described in the DGP treatment manual. This included an initial engagement phase with six one-hour group sessions conducted on a weekly basis. The groups were run by clinicians from community-based adolescent mental health services. Similar to the original Manchester study, adolescents who completed the six initial group sessions had the option to continue on with a more long-term group for up to twelve months. Adolescents in group therapy also received routine care from their local adolescent mental health service.

To make sure that the group therapy sessions were as close to the Manchester model as possible, a group therapist who had been involved in the original study helped to oversee the program and conducted 'booster sessions' as needed. Video conferencing also allowed the Australian therapists to consult with the British therapists about specific clinical concerns. All group sessions were videotaped and three British therapists rated them according to how closely they matched the DGP treatment manual.

The program's effectiveness was measured using assessments in four waves: immediately after being assigned to a treatment condition and then eight weeks, six months, and twelve months following assignment. Along with looking at whether or not there were any self-harm attempts, participants completed questionnaires measuring suicidal thinking, substance abuse, psychiatric symptoms, and overall level of functioning. Information on family issues, previous psychiatric history, home environment, and history of abuse was also collected by the researchers. The group therapy and control participants were fairly well-matched with no real differences that might distort the research findings.

According to the research results, 97% of adolescent self-harmers reported cutting themselves while 71% also reported head-banging. Deliberately overdosing on medication was the third most prevalent method (57%) while other forms of self-harm, including jumping from a height, attempted drowning, poisoning, and strangling were less prevalent. Some of the adolescents participating in the study reported cutting themselves as often as once a week or more.

While alcohol abuse was fairly common among self-harmers, drug abuse was not. Furthermore, half of all self-harmers live in two-parent households while about a third reported a history of sexual abuse. According to test results, there was no significant difference between the experimental and control group subjects in terms of depression, behaviour problems, or overall psychological functioning.

In examining how effective the group treatment program was in preventing self-harm attempts, the results failed to support the positive results of the Manchester study. If anything, more of the participants attending group therapy harmed themselves during the follow-up period than the control participants did. Participating in the group program also had no apparent effect on depression or suicidal thinking.

Taking the next step

So why didn't a group program that had seemed so promising when carried out by British researchers help Australian adolescents who harm themselves? In their article, Professor Hazell and his co-authors point out various differences between the adolescents in the two studies that may have played a role. Not only were there far more females in the Australian study than in the British study (91%) but they were more likely to be self-cutters as well. Also, the therapists conducting the groups in Australia had less experience with the DGP program than the British therapists who developed the program in the first place. This may have made them less effective in helping their patients.

'About one in ten adolescent females and one in twenty adolescent males engage in self-harm'

Despite these disappointing results, Professor Hazell and his co-authors raise one important point: many of the young people who harm themselves did it on a regular or semi-regular basis. Even if group therapy doesn't eliminate these attempts completely, they may make them less severe and encourage them to seek out help later on.

When asked about his opinion on treatment for adolescent self-harmers, Professor Hazell remains optimistic. 'As a clinician and medical administrator I am striving to develop better systems of care for young people who engage in self-harm', he says. 'My health service is presently bidding to be a site to evaluate a multi-component intervention to reduce self-harm and suicide. I am seeking ways to divert young people who self-harm away from hospital emergency departments. As a researcher I am still on a quest to identify an intervention that reduces repetition, or as I prefer to say it – hastens the attenuation of self-harm.'

Even though DGP remains unproven, at least in countries aside from where it was developed, further research may provide better clues about how to improve treatment services. While young people appear to be getting better at seeking treatment, more effective programs are still needed to ensure that children and adolescents find the resources they need to move on with their lives.

Meet the researcher

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Professor Philip Hazell is Conjoint Professor of Child and Adolescent Psychiatry at the Sydney Medical School at the University of Sydney, Australia. He is also Director of Child and Adolescent Mental Health Services for the Sydney Local Health District and Director of the Rivendell Child, Adolescent and Family Mental Health Service in New South Wales. After obtaining his medical degree at the University of Otago and training as a psychiatrist at the University of Adelaide, he went on to earn his doctorate in medicine at the University of Newcastle. His long list of publications includes research on ADHD, youth suicide and deliberate self-harm, mood disorders, autism, children in out-of-home care, systematic reviews on treatment effectiveness and the evaluation of medical education.
Professor Hazell was honored by the American Academy of Child and Adolescent Psychiatry with the Elaine Schlosser Lewis Award for Research in Attention Deficit Disorder in 2004. He is currently a co-investigator on funded longitudinal studies of children with Attention Deficit Hyperactivity Disorder and of determinants of health and wellbeing in adolescents in rural New South Wales. He is also a co-investigator on a funded clinical trial of fluoxetine for autism. Along with a lifetime of academic achievements, clinical appointments, and community service, he is also an accomplished violinist who has played in symphonies and an avid surfer.

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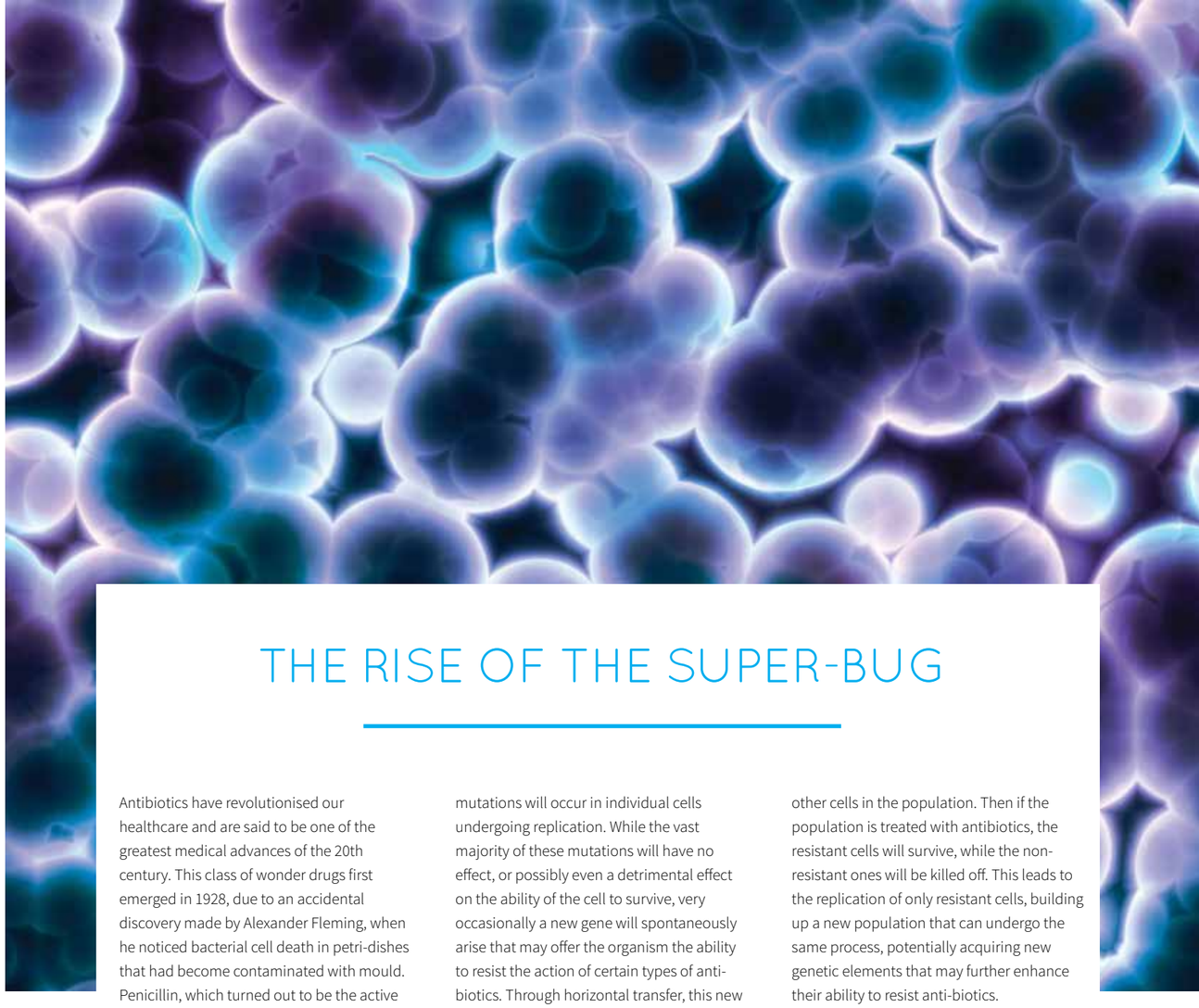


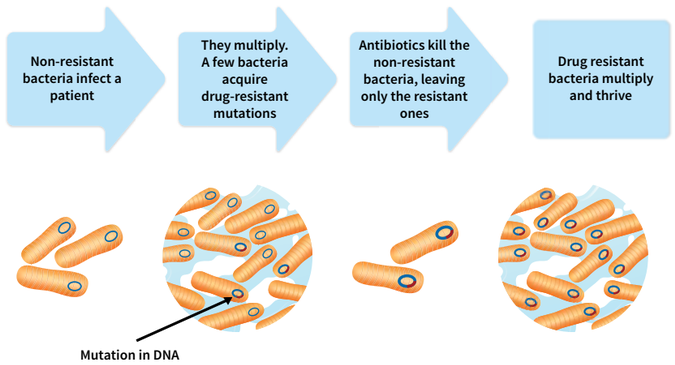
THE RISE OF THE SUPER-BUG

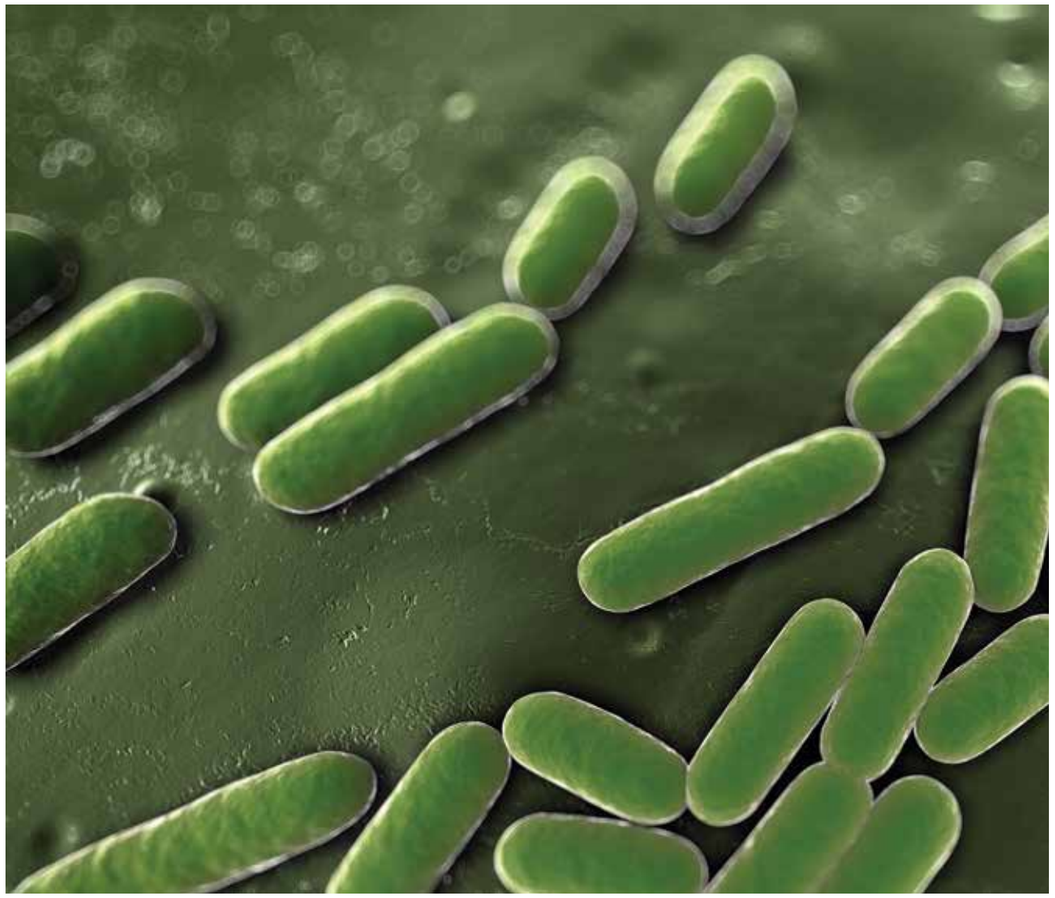
Antibiotics have revolutionised our healthcare and are said to be one of the greatest medical advances of the 20th century. This class of wonder drugs first emerged in 1928, due to an accidental discovery made by Alexander Fleming, when he noticed bacterial cell death in petri-dishes that had become contaminated with mould. Penicillin, which turned out to be the active compound produced by the blue-green mould, is still widely available today in a variety of different forms.

While animals can take thousands, or even millions of years before natural selection leads to noticeable changes in their genetic makeup, bacteria reproduce at an alarming rate, and thus under the right conditions, significant genetic changes in bacterial populations can occur in a matter of days. In addition to evolving through random mutation (as animals and plants do), bacteria can also acquire genetic variation through a process known as horizontal transfer, whereby bacterial cells pass DNA back and forth to one another. These phenomena have given rise to the emergence of anti-biotic resistance, and the rise of super-bugs, such as MRSA.

Take a population of non-resistant bacteria for example. During multiplication, natural mutations will occur in individual cells undergoing replication. While the vast majority of these mutations will have no effect, or possibly even a detrimental effect on the ability of the cell to survive, very occasionally a new gene will spontaneously arise that may offer the organism the ability to resist the action of certain types of anti-biotics. Through horizontal transfer, this new genetic information can be shared amongst other cells in the population. Then if the population is treated with antibiotics, the resistant cells will survive, while the non-resistant ones will be killed off. This leads to the replication of only resistant cells, building up a new population that can undergo the same process, potentially acquiring new genetic elements that may further enhance their ability to resist anti-biotics.

How Bacteria Develop Resistance

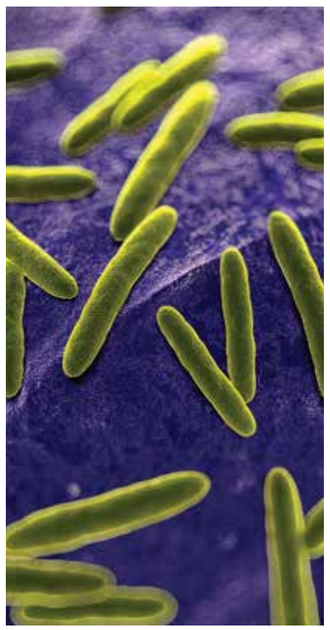




As we will see in this section, bacteria can even acquire new DNA from viruses known as phages. Understanding the mechanisms behind this process is one of the aims of Dr Patrice François and his team at the Genomic Research Laboratory in Geneva, Switzerland. The team perform genetic studies on Staphylococcus aureus, a common type of infectious bacteria. S. aureus bacteria started off as a harmless coloniser and evolved, through the insertion of viral genetic material, into the super-resistant, dangerous pathogen that it is today. Dr François' work can offer new insights into the mechanisms by which how bacteria mutate to become pathogenic, and how resistance to antibiotics arises. In this way the team can identify the origin of genetic transfers in order to limit or control further emergence of highly pathogenic bugs.

Next in this section, we introduce the work of Dr Clare Kirkpatrick, who conducts research into the molecular pathways within bacteria, by defining potential new targets and modes of activity for antibiotics. There is an urgent need to discover new potential targets and pathways in multi-drug resistant bacteria, in order to develop novel antibiotics. New molecular targets may also help to teach us how existing antibiotics can be used more effectively in different combinations.

Finally, we highlight the fascinating work of Professors Roger Koeppe II and Denise Greathouse at the University of Arkansas, USA, who design and study peptides that interact with model biological lipid-bilayer membranes. As part of their research, the pair is interested in membrane-peptide interactions and how this knowledge can be used to treat anti-biotic resistant strains of bacteria, as many membrane-active peptides and proteins have anti-microbial properties. This work may lead to the use of peptides as prototype molecules for the development of new anti-infection agents.



HOW MUTANT STAPH JUMPED FROM LIVESTOCK TO HUMANS

Dr Patrice François and his team at the Genomic Research Laboratory (GRL) have investigated a recently evolved staph strain that originates in farm animals and mutates into a pathogen adapted to humans. This pathogen can be found in human populations that have no direct contact with livestock.

Research into staph evolution

Dr Patrice François' interest in an infectious agent called Staphylococcus aureus (S. aureus) was stirred by the intricate circumstances surrounding the evolution of bacteria, and the medical potential of such discoveries. Describing the significance and scope of his research, François tells Scientia: 'Since 2000, we are using strictly the same protocol of survey for patients suffering bloodstream infections due to S. aureus. We noticed an evolution with the emergence of a specific clone of S. aureus responsible for severe infection in humans. Initially, this clone was restricted to pigs or pig farmers and was a poorly pathogen, meaning that it was mainly found in asymptomatic carriers. Suddenly, variants of this clone were found in severe infections in humans. The starting point of this evolution is, probably, industrial livestock. This evolution appears associated with the acquisition of mobile genetic elements by "naïve non-pathogenic strains" contributing to modify host tropism and to an increased pathogenicity in humans.' To test their working hypothesis, François and his team infected bacteria with viruses and followed its behaviour to see whether phenotypic changes occur.

How staph became infectious

Today, staph infection is synonymous with fever, cough, sinusitis, skin and soft tissue infection, foreign body infection, osteomyelitis or endocarditis. However, this has not always been the case. In fact, staph bacteria began as being a harmless colonizer and grew, by insertion of viral genetic material, into the super-resistant, dangerous pathogen that it is today. In this context, Dr François' work carries a lot of weight because it can offer new insights into the mechanisms of how bacteria mutate to become pathogenic, and how resistance to antibiotics arises. Moreover, such pathogens possess a high potential to resist treatment due to the presence of many different, fast mutating strains.

Following mutation, pathogenic bacteria that arise can further mutate, evolving into increasingly dangerous pathogens that cause severe illnesses. The path towards gaining a better understanding of the process resulting in new bacterial behaviour is twofold. Researchers are investigating the way by which bacteria acquire new genetic elements from viruses in their attempts to discover the exact evolutionary mechanisms that give bacteria their harmful characteristics. In Dr François' words, the aim of this research is 'to decipher the mechanisms of this evolution, identify the origin of genetic transfers in order to limit or control further emergence of highly pathogenic bugs.'

What makes the staph infections dangerous and, implicitly, opens up highly productive avenues of research is the symbiotic combination of viruses and bacteria. Viruses cannot reproduce by themselves, and do so by depositing genetic instructions into living cells, meaning that they are not technically life forms. However, viral genetic material present in infected bacteria can turn them into new strains transmittable with increasing ease at each new generation. Further to that, their characteristics become more harmful for human hosts.

Acquiring mobile genetic elements

Within the last twenty years, a strain that first colonised, and later infected, pigs and livestock, called S. aureus clonal complex (CC) 398, has evolved the ability to colonise and infect humans. S. aureus CC398 was already a worldwide problem for the farming industry. Now, since it has evolved and crossed over to humans, even people who have not had direct contact with livestock or farms have been found to have contracted the disease.

For a better understanding of the inner workings of the staph evolution by the insertion of genetic elements, a few observations are in order. A genome is the complete set of genetic material made up of DNA inside the nucleus of a cell, and is the target of this study, since it carries all the genetic information necessary for replication and evolution. Dr François studied how this subpopulation of CC398 bacteria has evolved to infect humans by acquiring mobile genetic elements from viruses. Bacteriophage viruses – or phage in short – are types of viruses that freely infect bacteria and use them to reproduce. Pieces of viral genome inserted and, later on, integrated into the genomes of the next generations of bacteria are called prophage. Interestingly, at the stage where the prophage genome is integrated into the bacterial genome, it is no longer harmful or disruptive to the functioning of the host.

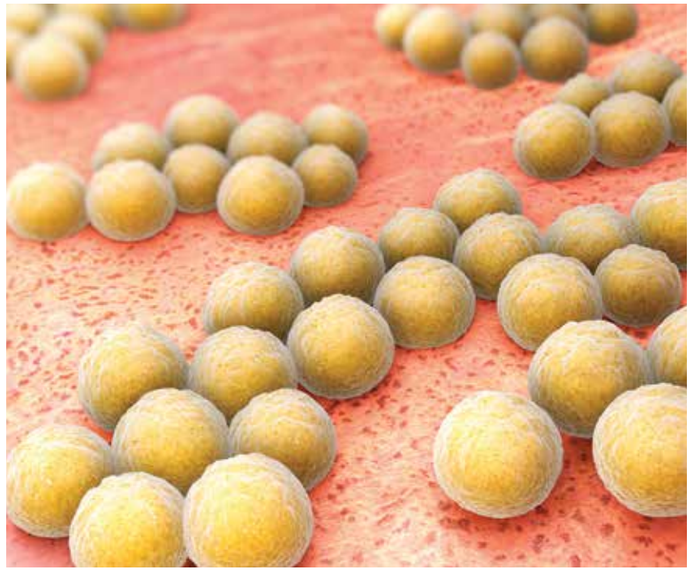
In the case of CC398, a genetic element was originally inserted by a virus into a bacterial cell and is now present inside the cell alongside the original CC398 genome. Since the prophage is actually the genetic material of the virus itself, it causes changes in the observable characteristics of the bacteria. A prophage can be integrated directly into the main bacterial genome, as is the case for CC398, and this type of phage is known as a 'lysogenic phage'. Prophages are a very important element of bacterial diversity and evolution. They are able to act as an evolutionary driving force for bacteria, allowing the development of new strains that are adapted to new environments.

Understanding pathogenic behaviour

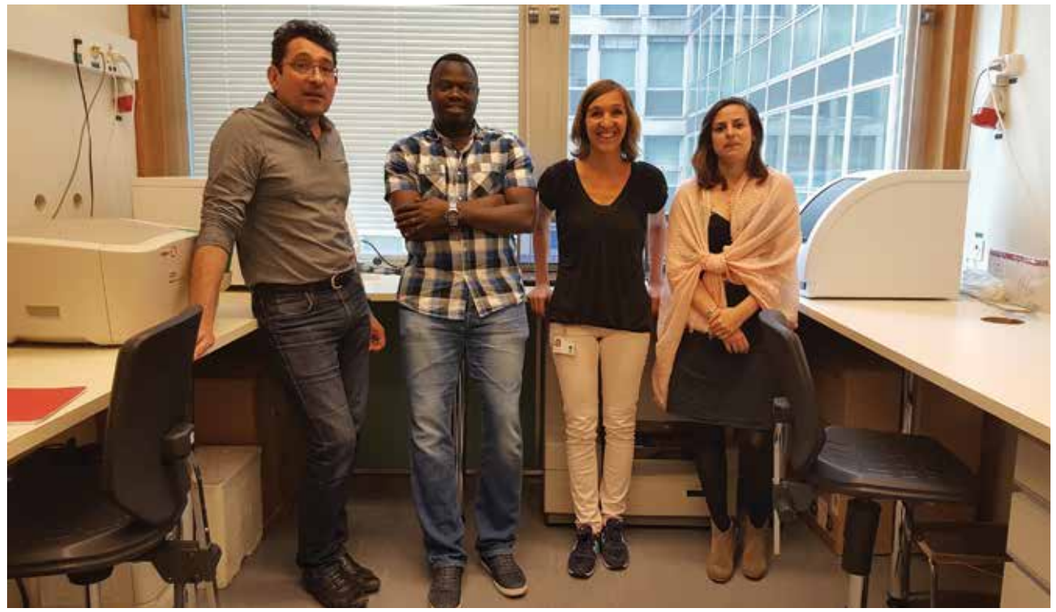
S. aureus is a bacterium that causes acute and chronic infections in humans and animals – in particular, infections of the skin and respiratory tract. Presently, there are many known strains of S. aureus, some of which do not respond well to classic antibiotics. In their recent work, Dr François and his team have used novel tools in genetics research, such as high-resolution whole-genome microarrays, prophage profiling, immune evasion cluster characterisation and whole-genome sequencing to investigate prophages in human-adapted CC398. With these tools they were able to study a bacteriophage virus (or phage) and two different prophages that are harboured by human-adapted CC398.

Under the influence of new mobile genetic elements, or from the interaction of the genotype with its surroundings, bacteria develop new observable characteristics and behaviours as part of the evolutionary process. While the genotype of an organism refers to its genetic constitution, the phenotype is synonymous with the set of new behaviours and interactions observed after infection. In order to study the process of phenotypic modifications by genotypic augmentation, the researchers inserted phages into isolates of CC398 that originally did not contain prophages, or in other words, isolates not yet adapted for becoming infectious in humans. One of the interesting phenomena they investigated was the range of effects that the phage's reproductive cycles have on the CC398 host. This was performed by studying a process called lysogeny, a phage reproductive cycle in which viral nucleic acid becomes embedded in the bacterial genome. By following the lysogenic cycle, researchers were able to investigate the resistance of CC398 to further phage infection, how CC398 evolves the ability to colonise human cells, and the virulence factors that are expressed by the phage genome once it is integrated.

'We noticed an evolution with the emergence of a specific clone of S. aureus responsible for severe infection in humans. The starting point of this evolution is, probably, industrial livestock'



Virulence factors are molecular by-products that arise due to instructions that are encoded in the phage genome which hijack the cellular machinery of the host cell. These molecules create favourable environmental conditions for the phage and, in turn, induce many of the symptoms experienced by the organism carrying the viral disease – in this case humans. In their research, Dr François' team infected staph with viruses, starting from the working hypothesis that bacteria became pathogenic after being infected by viral genetic material. The purpose of the experiments was to see how infected bacteria behave after infection, how the genome becomes fixed into the new form, and to



The team. from left to right: Patrice Francois, Seydina Diene, Eve-Julie Bonetti, Floriane Laumay.

study virulence factors induced by bacteriophage viruses into bacteria.

In the work, a total of 21 CC398 isolates were studied in two groups of livestock associated (LA) and non-LA bacteria. Dr François and his team found that in non-LA CC398, a prophage called StauST398-5pro offered significant protection of the host bacteria from horizontal genetic transfer to the host. Horizontal genetic transfer is a process where bacteria transfer genetic material directly to each other, which strongly contrasts with the normal parent-offspring means of genetic transfer. Horizontal genetic transfer includes the phage to prophage process that resulted in the evolution of the human-adapted CC398. The prophage StauST398-5pro was also found to influence virulence genes so that they are expressed in stress situations.

In 1961, an antibiotic-resistant strain of S. aureus was discovered, now known as methicilin-resistant S. aureus shortly after the introduction of the drug in human medicine. More prominently known by the acronym MRSA, today the resistant staph strain has become a global problem. MRSA evolved from a methicilin-sensitive predecessor that was not resistant to methicilin antibiotics, called MSSA. Of the 21 CC398 isolates studied, the majority of strains were MSSA, as opposed to MRSA.

Future research directions

Dr François' research does not end here, since there are many more important mechanisms to be unravelled. Many exciting avenues of research have been opened by the present work. Dr Francois tells Scientia that they 'are currently cutting these different mobile elements to identify the genes conferring new properties and phenotypes.'

Since S. aureus is a major source of infection for humans and animals, and is evolving into highly infectious and aggressive strains, it is highly important to understand the impact of mobile genetic element acquisition by bacterial genomes. Firstly, these elements produce notable changes in the invasiveness of bacteria, and secondly, the integrity of bacterial genomes has a heavy influence on their future evolution, behaviour, and resistance to antibiotics. Presently, this area of research is still novel and relatively unexplored, which leaves large gaps in our understanding and the ability to create sufficiently targeted antibiotics capable of addressing such infections.

The research led by Dr François next proposes to shed more light on this topic. The team is planning to discover unknown prophages, which confer bacteria important clinical features. By exploring the molecular mechanisms involved in the process that turns bacteria into pathogens, they seek a better understanding of cellular invasiveness. Identifying novel restriction mechanisms encoded by the genetic elements of bacteriophage viruses is another important point on their list, followed by assessing the regulation of the known restriction systems. How these changes become fixed within the genome by DNA methylation processes, and the identification of the exact biological functions encoded in the viral instructions are areas that will be targeted in future research.

'We are currently cutting different mobile elements (mainly composed of hypothetical proteins with unknown functions), to identify the genes conferring new properties and phenotypes'

All these aspects define the virulence, epidemiologic patterns, and pathogenic potential of future staph strains. For this reason, the team aims to decode the instructions carried by the phage genes – instructions which are responsible for the phenotypic changes observed in the latest bacteria generations. This is all the more important due to the increasing number of humanized strains observed in clinical settings.

Meet the researcher

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Dr Patrice François is a team leader at the Genomic Research Laboratory (GRL), a world class research centre in Geneva, where he has been instrumental in its creation and development. In 1996, François received his PhD from Paris XIII University, France, and continued as a post-doc within the Division of Infectious Diseases. His research on the epidemiology and the regulation of virulence factors in S. aureus has attracted multiple awards, such as the GlaxoSmithKline award (2004), the Pfizer award (2004), and the Sanofi-Aventis award (2006). The Faculty of Medicine of Geneva awarded him the Privat-Docent degree for his work on bacterial genome and virulence bases in methicillin-resistant staph, or MRSA. His work includes 181 published research articles and 17 book chapters, totaling a citation index of 49 and bringing him 6,789 citations.

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Genomic Research Laboratory

THE TOXIN-ANTITOXIN SYSTEM WITH A FEW TRICKS UP ITS SLEEVE

Dr Clare Kirkpatrick's research has led to the discovery of a bacterial toxin-antitoxin system with unique features. This information can be used in future studies to identify novel molecular pathways in bacteria which can be targeted by new antibiotics

Antibiotic resistance is a growing concern for microbiologists and poses an increasingly serious health problem globally. Following the discovery of antibiotics, starting with the isolation of penicillin in 1928 by Alexander Fleming, their use became increasingly widespread around the end of the second world war. The number of highly antibiotic resistant strains of bacteria has increased concomitantly ever since, leading to the emergence of MRSA, to give one example. Before doctors and scientists understood the potentially catastrophic consequences of their overuse, the use of antibiotics was largely uncontrolled until recently. In many parts of the world it remains unregulated, and antibiotics can be bought freely over the counter. The number of new antibiotic classes being discovered has also slowed down dramatically, with almost all of the antibiotics currently in use having been discovered prior to the 1990s. Without new classes of antibiotics, the risk of the emergence of pathogenic strains of bacteria which show resistance to all known antibiotics, becomes increasingly likely over time.

The issues surrounding antibiotic resistance are highly complex and require a sustained effort worldwide from politicians, the pharmaceutical industry and scientists in order to be resolved. Failure to solve this problem, in the worst case scenario, could lead to a situation where bacterial infections which would be considered trivial by today's standards, could become incurable and lethal in the near future. Even now, some hospital-acquired infections by the most resistant strains can fall into this category.

Scientists such as Dr Clare Kirkpatrick, who conduct research into the molecular pathways within bacteria, are at the forefront of this research effort, by defining potential new targets and modes of activity for antibiotics. 'Development of new antibiotics is unlikely to ever be a source of significant revenue for drug companies, very little industry resources have been focused on it and the rate at which new antibiotics come to the clinic is very low', Dr Kirkpatrick tells Scientia. She believes therefore, that academia is a more likely setting for generating knowledge in this area, rather than industry, which by its nature remains profit-oriented. She also believes that since the number of known antibiotic targets within bacteria is so low, new potential targets and pathways may need to be discovered before novel antibiotics can be found. New molecular targets may also help to teach us how existing antibiotics can be used more effectively in different combinations.

The HigBA toxin-antitoxin system

Working under the supervision of Prof Patrick Viollier at the University of Geneva, Dr Kirkpatrick helped to discover a novel regulatory pathway within *Caulobacter crescentus,* which was published in the prestigious journal, *Nature Microbiology.* The pathway involved a set of proteins constituting a 'toxin-antitoxin system' (TAS), in which a set of connected genes code for proteins which have distinct functions, one as a poison and the other as its antidote. The antitoxin part of the system generally works by binding to the toxin protein and inhibiting its function. Sometimes this might be solely to ensure that the two genes are passed on to the next generation, where a cell divides in two, with one new cell retaining genes for both proteins and the other losing them. In this situation, the toxin is generally more stable than the antitoxin in the new cell, so when the antitoxin eventually degrades, all that remains is the toxin and the cell is killed. Over the whole bacterial population, this ensures the proliferation of the two genes. Another purpose for TASs, is to cause

‘Antibiotic resistance is an insidious problem, sometimes described in a more sensationalist way as a “time bomb” or an “antibiotic apocalypse”.’

bacterial cells to enter a dormant state under stressful conditions. This means that the only remaining cells will be the ‘persister cells’ which tolerate the stress conditions or antibiotic in question without needing to acquire genetic resistance mutations. Scientists have been considering using TASs for some time, in the fight against antibiotic resistance, but depending on the specific TAS, the toxin may either kill the cell or place it into a dormant state, during which it is insensitive to antibiotics, even at high doses. When these cells awaken from dormancy, for example when a patient finishes a course of antibiotics, they are just as infective as before.

One such system is called HigBA, where HigA is the antitoxin, working by inhibiting the activity of the toxin and also by preventing the ‘reading’ of the gene which codes for it (known as transcriptional repression). The toxin, HigB, is a type of enzyme which targets and breaks down RNA, a molecule similar to DNA which is required for genes to be read by the cell and coded into protein.

To give an analogy for this process, if the genome (made up of DNA) is a library, then RNA could be thought of as photocopies of particular pages, giving instructions for building different things, which would be proteins. Each gene will produce specific RNA molecules which can be read by the cell’s protein-producing machinery. If the RNA is degraded, the protein cannot be produced. One of the key features of this particular system, which makes it such a novel discovery is that unlike previously described TASs, the HigBA system targets particular RNA molecules, representing specific genes, rather than just degrading every bit of RNA available. The system is also unique in that it appears to regulate the way the cell grows and divides (the cell cycle), and responds only to a particular and highly specific kind of stress: DNA damage.

HigBA and the DNA damage response

Before Dr Kirkpatrick and her colleagues learned how HigBA responds to the presence of DNA damage, they were looking in a different direction, at a mutated *Caulobacter* containing a defective gene for TipN. TipN is a protein involved in defining cell polarity (determining which end of the cell is the front and which is the back) by driving the placement of proteins essential for assembly of the flagellum (which could be described as the cell’s propeller, required for motility). These polarity mutants displayed the unexpected property of increased sensitivity to the antibiotic nalidixic acid. This antibiotic normally slows the rate of bacterial growth by inhibiting the cells from replicating their genomes, which is required prior to cell division. This seemed to happen in the TipN-defective mutant even though the known target of nalidixic acid in *Caulobacter* has a natural mutation making it resistant to it. The team discovered that this curious situation was caused by nalidixic acid dislodging a transcriptional repressor, allowing the expression of a particular efflux pump protein complex (the function of these proteins being to selectively pump specific molecules, such as antibiotics, out of the cell). The mutant lacking TipN did not tolerate increased expression of this efflux pump, for reasons that are still unknown. HigBA was found to be involved in this process, as the toxin, HigB, decreases the expression of the efflux pump, by specifically targeting its RNA and degrading it. If the antitoxin, HigA, is mutated to become non-functional, the toxin is set free and the cell’s resistance to nalidixic acid is partially restored. The toxin, therefore, provides increased tolerance to this antibiotic by reducing expression of the efflux pump, and actually promotes bacterial cell growth, rather than inhibiting it in this situation. Again, this is unusual as efflux pumps would usually confer resistance, by pumping antibiotics out of the cell, rather than increasing antibiotic sensitivity.

Interestingly, the opposite is true during the DNA damage response, where bacteria *without* a functional HigB toxin gene show increased resistance to DNA damage-inducing agents such as mitomycin C and ciprofloxacin. This was discovered by testing the TipN-defective mutant’s growth in the presence of a chemical library containing a wide range of antibiotics, to search for others which, like nalidixic acid, were specifically inhibitory for this mutant. Two other antibiotics with these properties were found, but surprisingly the HigB toxin did not protect against them. Because they both belonged to the family of quinolone antibiotics, that block DNA replication, these findings were also tested in cells which are mutated to constantly behave as they do in the presence of DNA damage, by mutation of a gene called LexA. This experiment showed that HigB was only protective if the antibiotic did not cause DNA damage; otherwise it contributed to cell death. Indeed, this is what makes the HigBA system unique. Other TASs seem to work in concert, responding to general stress to the cell, whereas the HigBA system seems to respond specifically to stress induced by DNA damage. The HigBA system is involved further still in the DNA damage response pathway. The LexA gene, it turned out, explained why it is even possible to produce mutants which lack a functional HigA gene. Usually, in a TAS such as this one, disrupting the function of the antitoxin element of the system leads to inevitable cell death resulting from unregulated activity of the toxin, which is pretty much the point of these systems. In the HigBA system, LexA binds to the HigBA gene in the same way that HigA does, by performing transcriptional repression. Only in the presence of DNA damage, or when both LexA and HigA are deleted, is the toxin fully derepressed and allowed to kill the cell.

The influence of HigBA on the cell cycle

Although HigBA certainly is involved in the DNA damage response, that does not appear to be its sole function, with the system also having a role in regulating the cell cycle. The cell cycle is the highly complex ‘program’, involving a large number of molecular pathways, which cells use in order to carry out the processes of growth, DNA replication and cell division in the appropriate order and at the appropriate time. While looking at the RNA being targeted by the HigB toxin, Dr Kirkpatrick and her colleagues found that in addition to the efflux pump RNA, HigB also targeted CtrA. CtrA is a regulatory protein involved in controlling the cell’s transition into the DNA replication stage of the cell cycle, necessary for subsequent cell division. Its function is to maintain the cells in the swimming stage of the cell cycle (in which they do not replicate their DNA) and clearance of CtrA out of the cell allows the DNA replication stage of the cell cycle to start. They found that when the antitoxin was defective, there were fewer CtrA-dependent swimming cells in the population. So when the HigB toxin levels are higher, its action against CtrA allows the cells to proceed more quickly to the DNA replication and division stages of the cell cycle. Again, this makes the HigBA system unique among TASs in its ability to fine-tune the

cell cycle, and it may further contribute to the DNA damage response since actively replicating cells are more sensitive to DNA damaging agents. ‘HigBA is highly specific both in its activation conditions and its response,’ explains Clare. ‘It is dedicated exclusively to the DNA damage response in these bacteria and attacks a small set of essential targets in the cell, leading to inescapable cell death.’

‘Discovery of new pathways within bacteria that could provide a source of new drug targets, as well as new molecules that interfere with them, is a challenge that can more easily be met in an academic setting without the requirement to generate profits for shareholders.’

The future

Dr Kirkpatrick’s new lab at the University of Southern Denmark will be focussed on chemical screening for compounds which inhibit the growth of *Pseudomonas* bacteria. *Pseudomonas* species can be disease-causing and are the second most widespread cause of hospital acquired infections. Antibiotic resistance is also known to be on the increase in this genus. This screening will be designed specifically to identify compounds which affect the pathways involved in determining cell polarity. Initial experiments performed in this screen, such as the one which identified additional TipN-specific inhibitor compounds, tested normal *Pseudomonas* cells against those which were defective (mutated) in genes involved in regulating polarity, expecting that the compounds of interest would inhibit cell growth in the mutants but have no effect (or less of an effect) in the normal cells. ‘I was very surprised to find that a few had the opposite effect,’ says Dr Kirkpatrick, ‘the mutation of the gene appeared to confer a protective effect on the cells against the antibiotic, even in cases where the gene in question was not known to be involved in the usual mechanism of the antibiotic.’ She plans to prioritise the study of these compounds as they may provide information on previously unknown pathways and drug targets. She also plans to use techniques which mutate large numbers of bacterial genes in order to find candidate genes which either protect or inhibit the bacteria in their resistance to potential antibiotics. This screen will be focused on finding genes which are involved in defining cell polarity, as her work on *Caulobacter* demonstrated this to be a promising avenue.

Meet the researcher

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Dr Clare Kirkpatrick's interest in antibiotic resistance began while studying vancomycin-resistant *enterococci* as an undergraduate at the University of Cambridge, UK. After completing her PhD in bacterial genetics at the University of Cambridge, she then carried out her research on *Caulobacter crescentus* as a post-doc in Patrick Viollier's lab at the University of Geneva and subsequently acquired a Swiss National Science Foundation/Marie Heim-Vögtlin career development grant to initiate an independent research project on *Pseudomonas aeruginosa* chemical genetics. She is now starting her own laboratory group as an assistant professor at the University of Southern Denmark, Department of Biochemistry and Molecular Biology in March 2017.

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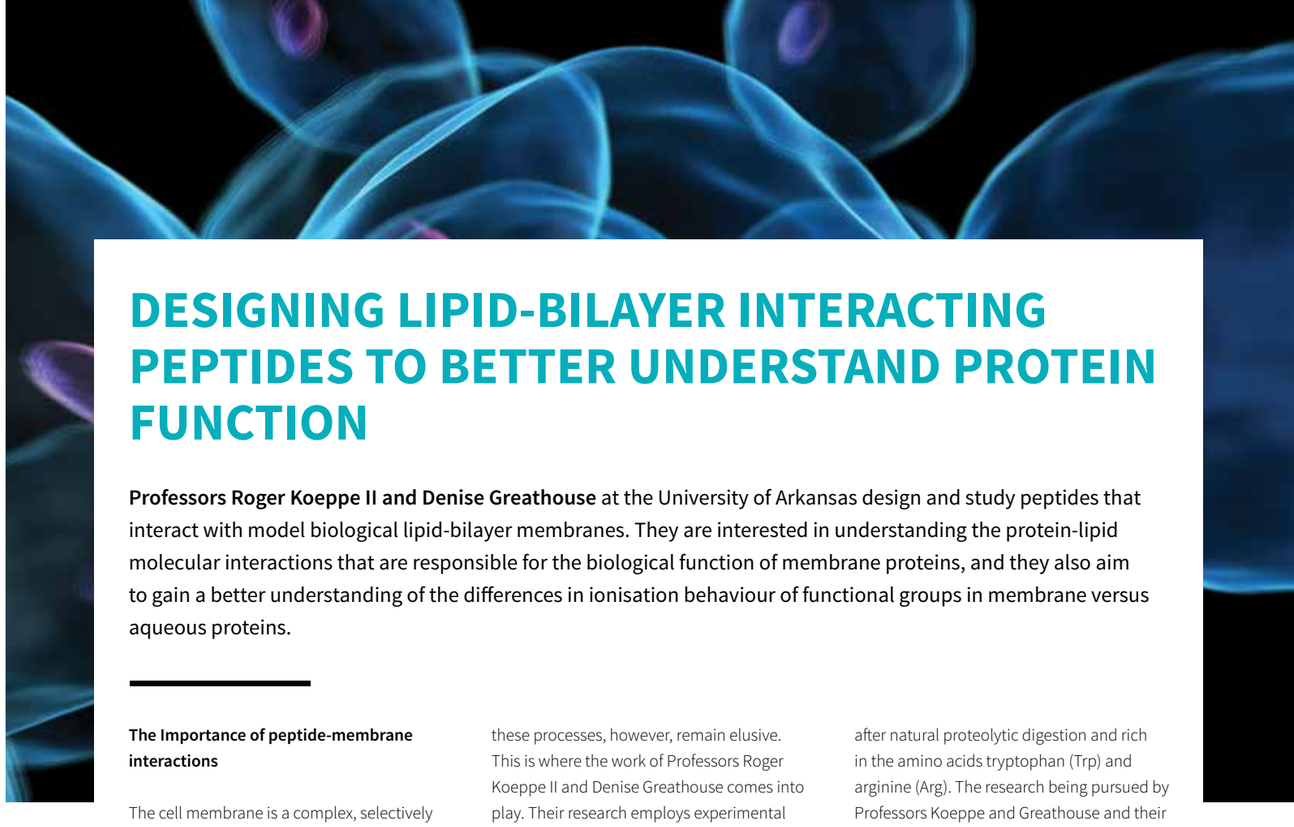


DESIGNING LIPID-BILAYER INTERACTING PEPTIDES TO BETTER UNDERSTAND PROTEIN FUNCTION

Professors Roger Koeppe II and Denise Greathouse at the University of Arkansas design and study peptides that interact with model biological lipid-bilayer membranes. They are interested in understanding the protein-lipid molecular interactions that are responsible for the biological function of membrane proteins, and they also aim to gain a better understanding of the differences in ionisation behaviour of functional groups in membrane versus aqueous proteins.

The Importance of peptide-membrane interactions

The cell membrane is a complex, selectively permeable, bilayer structure that separates the interior of cells from the outside environment. One feature of the cell membrane that is essential for numerous biological processes is its ability to interact with proteins. The building blocks of proteins are the 20 different amino acids, each having unique chemical characteristics such as size, solubility preference, and charge. Membrane interacting peptides are small proteins made up of less than 50 amino acids. They exhibit a wide range of biological activities and are involved in a vast array of cellular functions, such as antimicrobial defence mechanisms, membrane fusion, viral translocation and the transport of therapeutic compounds. Thus, due to their potential biomedical applications, membrane-active peptides are a major focus of much current research.

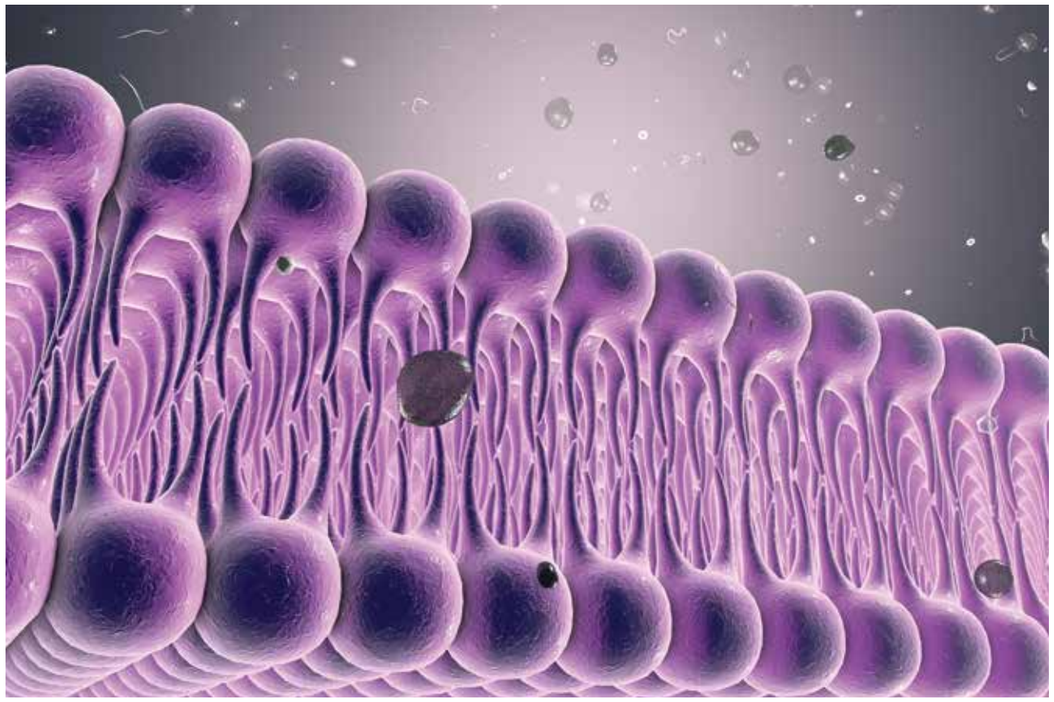
When peptide-membrane interactions occur, both the peptide and the membrane can undergo a series of conformational and/or structural changes. Researchers use both theoretical and experimental studies to gain insights into peptide-membrane interactions. This is a highly challenging field of research, with the overall goal of achieving a better understanding of the relationship between the amino acid composition of the peptide and the manner in which the peptide interacts with the membrane. Many of the molecular details of these processes, however, remain elusive. This is where the work of Professors Roger Koeppe II and Denise Greathouse comes into play. Their research employs experimental methods to focus on understanding the molecular mechanisms behind the specific interactions that determine how peptides that span and anchor to membranes interact with lipids and adjust their geometry in response to changes in the environment. The experimental results in turn can help to advance theoretical understanding and computational predictions.

As part of their research, the pair is interested in membrane-peptide interactions and how this knowledge can be used to treat resistant strains of bacteria. 'Membrane-active peptides and proteins are vital to many physiological processes, including signal transduction and ion conduction, and many have anti-microbial properties,' Professor Greathouse explains. There is an increasing occurrence of multi-drug resistant bacteria, in both the healthcare and community settings. Currently, there is a scarcity of new antimicrobial drugs to fight these bugs, and this poses a great threat to public health. Our first line of defence when our bodies are presented with a new pathogen, like one of these drug-resistant bacteria, is the innate immune system. This aspect of our immunity might be a rich source of prototype molecules (peptides) for the development of new anti-infection agents. Amongst these peptides are the mammalian lactoferricin peptides, derived from lactoferrin proteins after natural proteolytic digestion and rich in the amino acids tryptophan (Trp) and arginine (Arg). The research being pursued by Professors Koeppe and Greathouse and their team aims to understand the membrane interactions and mechanisms of action of peptides within the lactoferricin family.

Subtle peptide changes lead to great functional consequences

Amino acids are the building blocks of proteins, and peptides can be simply described as short proteins. The properties of amino acids contribute to the overall function of proteins and peptides. Lysine (Lys) and arginine (Arg) are both basic amino acids, and they possess important functions involved in membrane protein activity. These amino acids can sense membrane voltages and play roles in the actions of antimicrobial, toxin and cell-penetrating peptides. One key feature of amino acids that can be used to regulate protein function is the ionisation state. Atoms or molecules can become ionised by acquiring charge through the gain or loss of protons (hydrogen ions). When a peptide is trapped within the lipid-bilayer of the cell membrane, it is difficult to decipher the ionisation states of the individual functional groups. Professors Koeppe and Greathouse and their colleagues have addressed this issue for the functional components of the amino acids Lys and Arg in designed transmembrane peptide helices. In their method, they introduced a 'guest' Lys or Arg residue at a certain position in a

‘I remain fascinated by molecular function and the enormous variety of protein properties that can be realised using only twenty fundamental amino-acid building blocks’ – Professor Koeppe

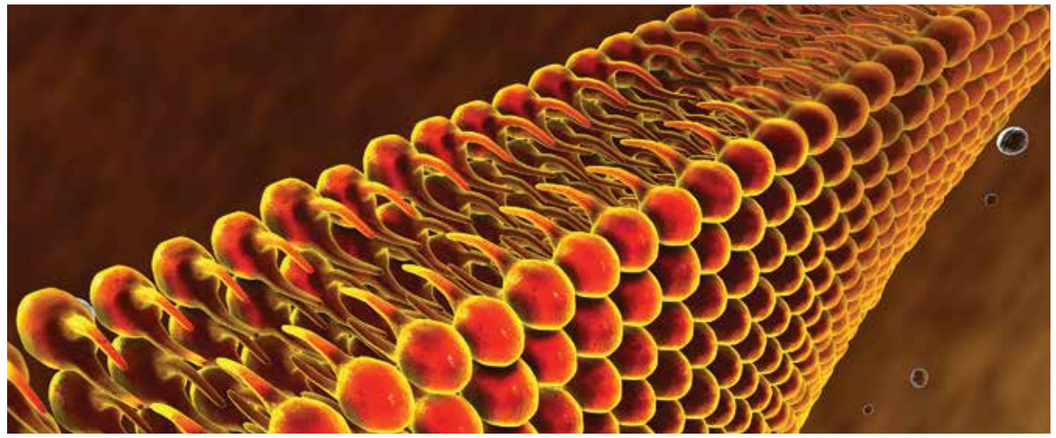


‘host’ membrane-spanning peptide. They found that in bilayer membranes at a low pH, when Lys is positively charged as a result of binding to a hydrogen ion, the Lys residues in the peptides behaved similarly to arginine. However, when the pH was elevated above pH 7, they observed that the Lys titrated (lost its charge) in the bilayer membrane, whereas the Arg remained charged. The researchers assessed the pH-dependent changes that occurred in the ‘tilt’ of the transmembrane peptide helix when each single Lys was titrated. They discovered that a buried charged Lys at certain positions within the transmembrane helix, like Arg, will cause the peptide tilt to change in order to maximise access of the charge to the aqueous interface. If, however, the charged residue is ‘sandwiched’ between neighbouring aromatic rings of Trp residues, which block access of the charges to the aqueous interface, the transmembrane peptide will leave the lipid bilayer. ‘One of the important results from this research is that the pH at which ionisable amino acids titrate (the pKa) when they are located in a membrane environment is very different from their pKa in an aqueous environment. Professor Greathouse explains, ‘These findings have important implications for understanding the mechanisms of large membrane embedded proteins such as those comprising the photosynthetic reaction centre and voltage gated ion channels, and are providing key information to facilitate methods that involve molecular dynamics simulations.’

Professor Koeppe describes the relevance of this work for biomedical applications: ‘Better knowledge of these fundamental molecular properties will lead to a better understanding of the mechanisms by which key signalling proteins function in biological membranes. Gaining greater insight into molecular function in turn leads to a better understanding of healthy and diseased states and may offer clues for new treatment options.’

Aromatic amino acids alter the orientation of peptides in the lipid bilayer

In their model peptides, Professors Koeppe and Greathouse include a core leucine–alanine sequence to promote the formation of a helical conformation and enhance the sensitivity of the peptide to the membrane thickness due to any hydrophobic mismatch between peptide and lipid. Aromatic amino acids are often located at the ends of transmembrane alpha helices of integral membrane proteins. Because of their preference for locations within the membrane–water interface of the lipid bilayer, the aromatic residues tryptophan (Trp), tyrosine (Tyr) and sometimes



phenylalanine (Phe) might function as anchors to help stabilise the transmembrane orientation. Therefore, the team compared the influence of Trp, Tyr and Phe amino acid residues at the membrane–water interface of the lipid bilayer upon the properties of tilted helical transmembrane peptides.

The team hypothesised that by altering the identities or positions of the aromatic anchors that surround the core sequence of a model peptide, the effects of these substitutions on the orientations and dynamics of the transmembrane helices could be investigated, and the results translated to better understand the behaviour of helices in membrane proteins. They discovered that when Trp, Tyr or Phe are replaced at the same position, one at a time within a model peptide, the peptide has the same average tilt and exhibits similar dynamics in the bilayer membranes. However, when two Tyr anchors are present, the model peptide exhibits more dynamic averaging and less responsiveness to the bilayer thickness than when two Phe anchors are present. Though Phe and Tyr are similar, it is notable that Phe lacks the hydrogen-bonding ability that is a crucial property of Tyr. These results suggest that subtle changes in the type of aromatic amino acid at the membrane-water interface can have dramatic effects on peptide helix rotation and dynamics and on the lipid membrane thickness. These effects may be important mechanisms that proteins use to regulate certain biological functions. The team concluded from this study that in the absence of other functional groups, aromatic residues at the membrane–water interface of the lipid bilayer determine the preferred orientations and dynamics of membrane-spanning peptides, which suggests possibilities for the rotational and dynamic control of membrane protein function.

A new way to study peptide helix stabilisation

The research conducted by Professors Koeppe and Greathouse has contributed to the notion that the dynamic properties of similar transmembrane helices can differ widely. While the exact mechanisms that direct the orientation or extent of helix dynamic averaging are not fully understood, it is clear that the type and location of amino acids, with slightly different properties, within the helix can have dramatic effects on the way the peptides interact with membranes, and therefore are important for defining the functional properties of membrane proteins. One possibility is that having ‘too many’ Trp or Tyr residues at the membrane–water interface of the lipid bilayer might correlate with high levels of dynamic averaging. The team sought to address additional factors, aside from the role of the Trp or Tyr aromatic rings at the membrane–water interface of the lipid bilayer, that could be responsible for stabilising the preferred orientations and limiting the dynamics of single transmembrane helices. To do this, they examined whether the amino acids at the ends of the peptide, outside the anchoring aromatic amino acids, might be unwound (frayed) when they are in the bilayer membrane environment. Indeed, the team observed substantial unwinding or fraying of the amino acids at the ends of several tilted helices spanning the membranes of the lipid bilayers.

‘One important result from our research is that the pH at which ionisable amino acids titrate when they are located in a membrane environment can be very different from when they are in water’ – Professor Greathouse

The observation of helix fraying led to speculation that hydrogen bonding between the unwound end segments and the membrane might be an important mechanism for reducing peptide dynamics and stabilising transmembrane peptides in lipid bilayers. As an example, it is already known that helix fraying is a mechanism used by multi-span proteins, such as G protein-coupled receptors, to adapt to a decrease in the hydrophobic thickness of the surrounding membrane. The model peptide–lipid systems designed by Professors Koeppe and Greathouse provide a novel way to address the intrinsic nature and potential significance of transmembrane helix fraying for membrane protein function.

Future Studies

The team is characterising the transitions of the amino acids lysine (Lys) and histidine (His) between neutral and positively charged states in several different lipid bilayer membranes. ‘Next we want to characterise the ionisation behaviour of glutamic acid (Glu) and aspartic acid (Asp) as they transition between neutral and negatively charged states in similar bilayer membranes.’ Professor Koeppe explains. ‘We also would like to investigate the influence of cholesterol on the ionisation properties that we measure.’

Meet the researchers

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Professor Roger Koeppe II is a distinguished professor in the Department of Chemistry and Biochemistry in the J. William Fulbright College of Arts and Sciences at the University of Arkansas. During his undergraduate studies in mathematics and chemistry at Haverford College, Professor Koeppe became intrigued with quantitative aspects of biological chemistry. From here, he then went on to receive his PhD from the California Institute of Technology, and did several years of postdoctoral work in Stanford. He has been recognised with an NSF Predoctoral Fellowship, an NIH Research Career Development Award, a Fulbright Fellowship (Netherlands, 1992), the Arkansas Alumni Research Award and the Fulbright College Master Researcher Award.

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Professor Denise Greathouse is an Associate Professor in the Department of Chemistry and Biochemistry in the J. William Fulbright College of Arts and Sciences at the University of Arkansas. Professor Greathouse's journey to becoming a peptide chemist began when she was a lab manager at the University of Wyoming, using one of the first fully automated commercial solid phase peptide synthesisers, the Applied Biosystems 430A. Her research now focuses on the synthesis of peptides designed to interact with cell membranes. Professor Greathouse has been recognised with the Faculty Gold Medal for undergraduate mentoring at the University of Arkansas.

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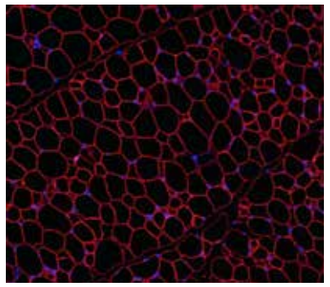
THE COMPLEX INTERPLAY BETWEEN NUTRITION, METABOLISM AND HEALTH

You are what you eat. A glib cliché? Perhaps, but our health, outward appearance and life span are, in large part, undeniably determined by our diet and the way in which we use the food we eat for energy. A multibillion-dollar weight loss industry and the multibillion-dollar healthcare costs associated with the myriad health conditions caused by obesity, are testament to modern society's unhealthy relationship with food. It seems that online purveyors of popular culture and tabloid media play a significant role in disseminating the work of nutritional scientists to a large audience, dictating which foods are 'bad' and which are 'good', and the faddy nature of the same, means that such results are often misrepresented, presented out of context or drastically exaggerated. At Scientia, we strive to disseminate research in an accurate and balanced way, let our featured researchers do the talking and let our readers draw their own conclusions. Significant research efforts are currently being devoted to understanding the nature of nutrition and metabolism and how we can leverage this knowledge to maximise health and prevent illness.

In this section we feature the work of three researchers. Our first article covers the work of Dr Philipp Scherer of the Touchstone Diabetes Centre, Dallas, Texas. Dr Scherer studies the interactions of adipose tissue with other organs, with a view to determining the impacts it has on health, and specifically looks at a molecule exclusively secreted by adipocytes, adiponectin, which he discovered. Our next article features the work of Dr Frances Sladek, of the University of California, who studies the effect that soybean and coconut oils have on health and looks at the mechanisms underlying these effects. Finally, we look at the research of Dr Linda J. Wykes and Dr Thomas Schricker, both of McGill University, who study the potential for nutritional care to enhance post-surgery recovery. They have found that appropriate nutritional care will reduce muscle wastage and improve immune function after surgery.

ADIPONECTIN: THE BODY'S FAT CONTROLLER

Dr Philipp Scherer's lab has been studying the role of fat cells in our metabolism. Over the past 20 years he has helped to explain how not all fat is created equal, and that the signals it produces influence the health of every other part of the body.



Fat cells (adipocytes) are something most of us feel like we have too many of and would be happy to see the back of. They represent the net difference between our energy intake and expenditure, so in evolutionary terms, adipose tissue is extremely useful, allowing the body to adapt to the changing availability of food and giving us metabolic flexibility. However, modern diets combined with sedentary lifestyles can often cause adipose tissue to accumulate in excess, which is associated with a wide range of health problems including diabetes, heart disease and cancer. Contrary to popular belief, these cells do not simply constitute a reservoir of excess fat. Since the early 1990s, scientists have learned that these cells are far from simple, with adipocytes displaying highly active and complex metabolic response as well as secreting a multitude of signalling molecules, affecting virtually every tissue in the body.

Dr Philipp Scherer has been researching the intricate details of how adipose tissue interacts with other organs for the last 20 years. His laboratory studies (among other aspects of fat cell function) a particular molecule which is secreted exclusively by adipocytes, called adiponectin. He discovered this molecule in 1995 and since then, adiponectin has been the subject of over 10,000 scientific studies. Broadly speaking, this protein influences various metabolic processes and protects cells from inflammation and apoptosis (programmed cell death).

Adiponectin, fat cells and metabolic health

Despite the specific nature of its fat-cell source, if you thought more fat means more adiponectin in the blood, you would be wrong. Counter-intuitively, the more fat you have, the less adiponectin will be in your system. In fact, adiponectin levels are negatively correlated with various metabolic disorders and diseases related to obesity. You might now be thinking, 'great, we clearly all need some more of this!', but again, biology often has a tendency to do the opposite to whatever common sense might suggest. When Dr Scherer's lab used genetic engineering to produce high quantities of adiponectin, regardless of weight gain (where in an un-modified mouse, levels would decrease), they might have expected that the mice would find it more difficult to maintain the weight gain. Instead the number of adipocytes skyrocketed, producing the fattest mice ever recorded, roughly equivalent to an 800lb (363kg) human. Yet, despite the obesity, these mice were very healthy and not diabetic!

Some of these contrary observations can be explained by the fact that not all fat tissues are equal. Adipose tissue can be made up of metabolically healthy fat cells, or dysregulated and metabolically unhealthy fat cells, depending on the manner in which the tissue expands. Our metabolic health is not always related to how obese we may be, but how sensitive we are to insulin, which is a pancreas derived hormone, produced in response to high blood sugar levels. Insulin regulates blood sugar levels by signalling cells to start taking up sugar to be used for energy. Resistance to insulin makes it harder for the body to regulate blood sugar levels. This resistance develops progressively and would eventually be diagnosed as type II diabetes. As Scherer's lab found out, insulin sensitivity is closely related to the healthiness of the adipose tissue.

As fat pads expand, blood vessels must develop with them, to provide oxygen. Hypoxia, a local insufficiency in oxygen supply, results from the failure of blood vessels to keep up with the expanding tissue, leading to various problems in its development. Hypoxia-triggered fibrosis causes increased cellular stress, and tissue necrosis (dead cells). The remnants of dead adipocytes attract white blood cells (macrophages) to clean up the mess, but this induces tissue inflammation. This inflammation, if left unresolved, leads to insulin resistance. Counterintuitively, treatments aimed at reducing inflammation do not appear to have much benefit in improving sensitivity to insulin. As Dr Scherer points out, 'adipose tissue is the biggest endocrine gland we have. It releases many protein factors, most of which are dysregulated in the obese state'. Metabolic health, the ability to tolerate high sugar or high fat diets, is therefore associated with healthy adipose tissue. The mice mentioned previously, with artificially increased adiponectin levels, displayed low levels of fibrosis in their fat tissue, as well as improved vascularisation, compared to obese, yet metabolically unhealthy mice.

White, brown and beige fat

In addition to metabolically healthy and unhealthy adipose tissue, fat cells can come in several different forms. The classical white adipocyte is the most common type of fat cell, but brown adipocytes also exist, which

'Despite our familiarity with fat tissue, there is a huge cloud of mystery around it'

are specialised in heat generation and are activated by cold temperatures. A third class of adipocyte, termed 'beige adipocytes' are located within white fat tissue, but possess the characteristics of either white or brown fat, depending on whether they are active or dormant (activation being triggered by low temperatures). The work of Dr Scherer's lab on adiponectin has identified a role for the protein in the differentiation of these fat cells. They have found that adiponectin is actually one of only a few signalling factors which appears capable of inducing 'beiging' of white adipose tissue. This may help to explain the observation that people with lower body-mass indices appear to display a higher proportion of brown fat.

Ceramides and how adiponectin influences insulin sensitivity

One of the primary functions of adiponectin appears to be sensitizing the surrounding tissues to insulin. In type I diabetic patients, for example, adiponectin levels appear to be increased, most likely to compensate for the diminished insulin production. Levels of adiponectin are also higher in metabolically healthy obese people compared to people with comparable body-mass indexes who are metabolically unhealthy.

Insulin sensitivity is impaired by a class of sphingolipids (a type of fat molecule) called ceramides, which are found in cellular membranes and block a particular part of the molecular pathway required for insulin sensitivity. Using genetically modified mice, Dr Scherer's laboratory studied the effects of lower ceramide levels on the liver, by over-expressing acid ceramidase, an enzyme which breaks down ceramide. These mice displayed an increased sensitivity to insulin. This improvement was true not only for the liver, but for the adipose tissue as well, effectively improving insulin sensitivity throughout the whole body. In addition to insulin resistance, ceramides have been linked to inflammation and apoptosis. Another important role of adiponectin is to decrease the levels of ceramide in various tissues, including the liver and heart, and certain anti-diabetic drug treatments which produce an increased level of adiponectin appear to cause an associated decrease in ceramide levels.

Adiponectin's tissue-specific effects

Dr Scherer's lab has studied the influence of adiponectin in various tissues, again by using a system of genetic modifications in mice, which allow them to induce apoptosis in particular target cell types, and then study the effects of increasing adiponectin concentrations on cell survival. These mice are called ATTAC mice, for Apoptosis Through Triggered Activation of Caspase 8 (caspase being a key protein in the triggering of programmed cell death). Since adiponectin levels are inversely associated with heart attack risk and coronary heart disease, Dr Scherer's system was used to study the effect of adiponectin on cardiac myocytes (heart muscle cells). These so-called 'Heart-ATTAC' mice show an increased rate of survival based on their levels of adiponectin. Similarly, their research shows that mice are more vulnerable to cardiac injuries if their ability to produce adiponectin is removed via genetic modification, but also in the absence of receptor molecules required for adiponectin binding to cardiomyocytes.

Heart cells are only one of many cell types that Dr Scherer's group have studied in this way. The system was also applied to the insulin producing ß(beta) cells found in the pancreas ('PANIC-ATTAC' mice), which showed a similar outcome to the Heart-ATTAC model – increased ß cell survival with increasing levels of adiponectin. Comparable outcomes were also observed when they performed the study on podocyte cells of the kidney ('POD-ATTAC' mice), which showed improved kidney function in mice with elevated adiponectin levels.

Dr Scherer's laboratory has shown that adiponectin not only increases insulin sensitivity, but also works with insulin to lower blood glucose levels by inhibiting glucose production by the liver. Adiponectin has a similar effect on fat production by the liver, by improving the metabolism and fat storage capabilities of adipose tissue.

'We need to understand what the role for the different fat depots is with respect to their endocrine and paracrine contributions'

There is a strong association between detectable adiponectin in the circulation and the incidence of a number of different cancers, with low levels being linked to breast, endometrial, colon and kidney cancers, as well as certain forms of leukemia. These observations are made at the epidemiological level, looking at large numbers of people. At the cellular level, increased adiponectin can actually be associated with increased tumour growth, possibly due to its angiogenic (blood vessel growth inducing) properties, as tumours, like an expanding fat pad, require vascularisation in order to avoid hypoxia.

Due to the role of adiponectin in so many

illnesses, metabolic disorders and insulin sensitivity, the receptors to which the protein binds are a source of great interest as potential drug targets, with the work of the Scherer lab and others being at the forefront of research into this area.

Uridine regulates the fasting response

One of the clearest benefits of fat tissue in the body is its ability to provide energy in times of fasting, such as by releasing free fatty acids as and when they are required. Other key nutrients are also released from adipose tissue, dependent on the metabolic situation, and one such nutrient, or metabolite, has been studied by Dr Scherer and his research group. That metabolite is called uridine, which is required for a diverse range of biochemical reactions, as well as being a key building block of RNA, which is required for the 'reading' of DNA. Uridine can even be used as an energy source, by neurons in particular. Normally, when we are not in a fasted state, uridine is synthesised by the liver. Dr Scherer and his team have discovered that when fasting, the liver ceases to produce uridine, which is instead provided by adipocytes, and the liver concentrates on making glucose instead, using the fatty acids released from fat tissue as an energy source.

Interestingly, uridine levels in the circulation appear to increase during fasting and decrease again after eating. Dr Scherer's group noticed that in metabolically dysfunctional mice, uridine levels are higher in general, and that their core body temperature is a few degrees lower. These differences are even more pronounced in these mice during fasting, and it turns out that one is responsible for the other. When these mice are injected with uridine, their body temperatures drop by several degrees temporarily. Scherer suggests that fat tissue may be regulating body temperature via its uridine synthesis during fasting, when a drop in body temperature is important for reducing the metabolic rate and preserving energy. This effect appears to be entirely independent of brown or beige fat cells. The group found that glucagon, another pancreas-derived hormone with the opposite effect of insulin (it increases blood glucose levels in response to fasting), is produced when the pancreas detects high levels of uridine. Scherer suggests that taken together, these observations indicate that fat cells use uridine as a core regulator of the fasting response.

The next steps

There are still many questions to be answered regarding the role of adipose tissue on regulation of metabolism, but it is clear that functionally healthy fat is about more than just obesity. Fundamental questions about the function of adiponectin still need to be answered, such as what signals adipocytes to produce it in such high levels? Dr Scherer explains that the future work of his lab will involve 'exploring fat tissue not only as a source of hormonal factors and lipids, but increasingly as a source for critical metabolites that the fat cell contributes to the system in times of need'. Future work will also focus on the role fat tissue has to play in cancer and other diseases. 'I believe we can expect many more surprising discoveries from the adipocyte in future', says Scherer.

Meet the researcher

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Dr Philipp Scherer is Professor of Internal Medicine and Director of the Touchstone Centre for Diabetes Research at the University of Texas Southwestern Medical Centre in Dallas. Dr Scherer performed his undergraduate and graduate studies in Biology and Biochemistry at the University of Basel in Switzerland in the 1980s and 1990s. He is best known for his discovery of adiponectin in 1995 and over the course of his academic career he has published over 320 scientific papers, some of which have been cited over 1000 times. He was recently awarded the American Diabetes Association's Banting Medal for Scientific Achievement in 2015 though his career has been distinguished with various other honours and awards.

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Close collaborators include colleagues in the Touchstone Diabetes Centre as well as other members of the metabolism group at UT Southwestern Medical Centre, such as Drs Roger Unger, William Holland, Rana Gupta, Olga Gupta, Joel Elmquist, Jay Horton and Perry Bickel.

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TURNING THE TABLES ON ‘HEALTHY’ FATS

Dr Frances Sladek is a world leader in molecular biology research. Here she talks to us about her latest research, how soybean oil affects health and what the molecular mechanisms that underlie these outcomes may be.

What is your research background and what triggered your interest in studying the effects of diet on metabolic health?

I was trained as a biochemist, doing my undergraduate work at Princeton and graduate work at Yale University. I became fascinated by molecular mechanisms and structural aspects of proteins, especially protein-DNA interactions. At the same time, due to illness in my family and friends, I became fascinated by cancer and its root causes, which is why I did my PhD dissertation on DNA repair and mutagenesis in bacteria. At the time, in the early 1980s, we didn’t really have the tools to do mechanistic work in mammalian cells and I figured that we had to first figure out how bacteria repaired damaged DNA before we could hope to figure it out in mammalian systems. My postdoctoral work at Rockefeller University took me initially away from the cancer focus as I purified and cloned a transcription factor, HNF4a, which is now considered to be a master regulator of liver-specific gene expression. HNF4a is a member of the nuclear receptor superfamily of ligand-dependent transcription factors such as the Vitamin A and D receptors, the oestrogen, progesterone and glucocorticoid (stress hormone) receptors. Around the time I cloned HNF4a, several other oprhan nuclear receptors were being cloned by other groups. Several of those receptors, and later HNF4a, were found to have fatty acids as ligands, including fats from the diet like linoleic acid.

What effect can polyunsaturated fatty acids such as linoleic acid (LA) have on health?

As an unsaturated fatty acid, LA in the past was generally assumed to be beneficial for health. There are, for example, reports on using LA to help prevent coronary heart disease. However, there are also a growing number of studies indicating that LA can have detrimental effects, such as insulin resistance and obesity. Nonetheless, compared to saturated fats which have been very well studied, since LA was found to be an essential fatty acid in the late 1920s, it has only been in the last five to ten years that researchers have begun looking at potential negative effects of LA.

LA is the precursor to arachidonic acid (AA), which is linked to inflammation, a key player in obesity, diabetes, inflammatory bowel disease and cancer so high amounts of LA could have detrimental effects compared to the relatively low amounts that are required for its essential functions.

What were the major differences between the diets high in soybean oil versus diets high in coconut oil?

The diet enriched in soybean oil led to obesity, diabetes, insulin resistance and a fatty liver phenotype with large lipid droplets and hepatocyte ballooning, a sign of liver injury. In contrast, an isocaloric diet in coconut oil led to relatively little weight gain, considering the amount of fat in the diet, no diabetes or insulin resistance. There was lipid accumulation in the livers of the coconut oil fed mice but much less than in the soybean oil fed mice.

How can these results be applied by healthcare professionals in their practice?

My recommendation is to try to avoid soybean oil. This is not easy to do as soybean oil is ubiquitous in the American diet. It is found in many, if not most, processed foods and vegetable oil, the main cooking oil in the US, is comprised of a mix of oils, one of which is almost always soybean oil. Restaurants in the U.S. use vegetable oil so if you eat out or eat pre-packaged food you are very likely consuming soybean oil. Even Jiff peanut butter has soybean oil on its list of ingredients! We never use vegetable oil at home but I have started adding coconut oil to my diet. We also stay away from processed foods but we do eat in restaurants. I should also add that I use soymilk every day in my tea or coffee and I love tofu. Soymilk and tofu do not have a lot of soybean oil in them so I would not recommend that one has to avoid all soy products.

What impact do you think this study and similar research could have on obesity prevalence?

According to our mouse studies, everything else being equal, if we cut down on the consumption of soybean oil as a society in general then I think we will see a decrease in obesity. A study in 2011 showed that the component of the American diet that has changed the most in the past 100 years is soybean oil, more than fructose, corn oil or chicken. Even though soybeans have been cultivated for 5000 years, we are now consuming more soybean oil than we ever have in the past.

ALL FATS CREATED EQUAL?

For decades, health authorities have been advising us to swap saturated fats for unsaturated. However, diabetes and obesity rates continue to rise. **Dr Frances Sladek** and her team in the University of California, Riverside ask if polyunsaturated fats are not as healthy as they once seemed.

Is Obesity Down to the Oil We Use?

It is no secret that obesity is on the rise in the Western world. There has been a steep increase in obesity in the last forty years, with 36 per cent of the US population and 26 per cent of the UK currently obese. This figure is expected to rise to up to 60 per cent by 2050. Obesity is associated with a number of conditions, including high blood pressure, heart disease, diabetes and insulin resistance, which together make up a disease known as metabolic syndrome. Of course several factors can be attributed to this increase: lifestyle, genetics, activity levels and environment, just to name a few. Diet, however, remains one of the key contributors to rising obesity. Recent years have led to a dramatic increase in fructose consumption, predominantly in the form of high fructose corn syrup which is found in soda and processed foods. Fat, and particularly the type of fat, has also been extensively scrutinised.

First, let’s get back to basics. There are three main types of dietary fats: saturated, monounsaturated and polyunsaturated. These simply refer to the types of bonds within the molecule of fat. Saturated fats are solid at room temperature and include butter, margarine, coconut oil and palm oil. Monounsaturated and polyunsaturated fats are liquid at room temperature. Monounsaturated fats include olive oil, canola oil, avocados and nuts. Polyunsaturated fats include soybean oil, corn oil and sunflower oil.

After a study in the 1960s which showed an association between saturated fats and cardiovascular disease, there was a significant increase in the consumption of soybean oil, found in many vegetable and seed oils, processed foods, salad dressings, snack foods and fast food meals. However, there appears to be a correlation between this increase in soybean oil consumption and the rising rates of obesity. This may be due to the linoleic acid (LA) contained within soybean oil, which is 55 percent LA. LA is an essential fatty acid that animals cannot produce so it must be consumed. However, LA needs to be only 1 to 2 per cent of our diet and we are currently consuming closer to 8 to 12 per cent. LA binds to a nuclear receptor called HNF4a, which regulates genes involved in carbohydrate and lipid metabolism and has been linked to both diabetes and fatty liver.

On the flipside, certain saturated fats such as coconut oil and palm oil may be useful in preventing and treating metabolic syndrome because they are full of medium chain triglycerides. These medium chain triglycerides are low in LA and are metabolised more quickly than other fatty acids and therefore contribute less to weight gain.

Metabolism at a Genetic Level

In order to examine the effects of unsaturated fats, as well as fructose, on obesity and diabetes, an experiment was designed which assigned mice to one of four diet groups. All diets contained the same amount of calories and each had 40 per cent total calories from fat, which is comparable to the average US diet today. The first high fat diet was made up of coconut oil, which was chosen because it is naturally low in LA and polyunsaturated fatty acids. The second group was a high fat diet in which ~50 per cent of the coconut oil was replaced with soybean oil to give 10 percent LA, comparable to the average US intake. The third group was a high fat diet with added fructose and the fourth was a high fat soybean oil enriched diet with added fructose. As well as the four diet groups, there was also a low fat control group in which the mice were fed a standard diet. Carbohydrate and protein levels were consistent across all diets and food intake did not differ significantly across groups of mice.

The results after 20 weeks are surprising. Overall, the diet high in soybean oil was the most detrimental to the health of the mice. This group gained almost 25 per cent more weight than the coconut oil group. This group also gained weight faster and had markedly more subcutaneous fat than the other groups. Very severe non-alcoholic fatty liver disease was also observed in this group. Researchers found very large fat droplets in the livers of these mice, as well as ballooning of the liver cells.

The soybean oil group also had significantly higher rates of diabetes, glucose intolerance and insulin resistance than the other groups, even when compared to the diets supplemented with fructose. This is particularly unexpected considering the links between high fructose diets and insulin resistance. Conversely, the coconut oil diet showed no signs of diabetes or glucose intolerance or insulin resistance at any time during the experiment.

Furthermore, while the fructose plus coconut oil diet did not cause diabetes, other health problems such as rectal prolapse and fatty liver were observed in these mice. While fructose had been linked previously by many

other groups to fatty liver, the prolapsed rectum finding was new but perhaps not surprising. Dr Sladek explains: 'The main negative effect of the high fructose diet that we did not observe with the soybean oil was a high rate of prolapsed rectums, which is part of the disease index of inflammatory bowel disease, which is on the rise. Fructose-free diets have been found to relieve gastrointestinal problems in humans.'

An analysis of the chemical processes involved in metabolism revealed an increased accumulation of polyunsaturated fatty acids and their metabolites in the livers of the soybean oil mice. Further study into the gene activity of the liver showed altered expression of a number of genes involved in metabolism, obesity, diabetes, inflammation and cancer. 'Some genes increased in expression while other genes decreased. There were several genes associated with obesity, diabetes, inflammation, and cancer that were up-regulated while some anti-cancer genes were down-regulated', Dr Sladek explains. 'But the largest category of genes that were altered were those involved in xenobiotic and drug metabolism. These are cytochrome P450 genes, many of which are known to be regulated by HNF4a and other nuclear receptors and which play an important role in metabolising not just toxic compounds that we ingest in our food and the drugs found in our pharmacy but also the steroids, retinols (Vitamin A compounds) and fatty acids that act as ligands for other nuclear receptors'.

'Fructose induced additional weight gain in the mice fed coconut oil but actually produced less weight gain than the mice fed soybean oil. This was rather surprising given the considerable attention given to high fructose corn syrup in the diet… In terms of the other tests, soybean oil, but not fructose, induced glucose intolerance and fructose actually lowered the insulin resistance induced by the soybean oil.'

Next Steps in Solving Soybean Oil

Studies like these are the only the first stage. Although the amounts of fat and fructose used in the experiment are similar to the average American's plate, these findings must be validated in humans before they can inform policy.

Dr Sladek and her team also want to find out the specifics of why soybean oil is causing such negative health effects. As Dr Sladek states: 'LA seems to be involved but we don't yet know if it is LA itself or if it is a metabolite of LA. We also would like to determine whether the negative effects of soybean oil are acting through HNF4a or other nuclear receptors that are also known to bind LA. Our results that we are submitting for publication now suggest that HNF4a and LA metabolites may indeed be very important in the obesogenic effects of soybean oil.'
So what's the takeaway? The shift towards soybean oil consumption may have improved cardiovascular health but also appears to have aggravated a host of other problems associated with metabolic syndrome. While the study doesn't indicate that diets high in fructose and saturated fat are the healthy option, it does show that a diet enriched with soybean oil can lead to a number of issues. This is an important factor for patients and healthcare providers alike to consider when planning a diet.

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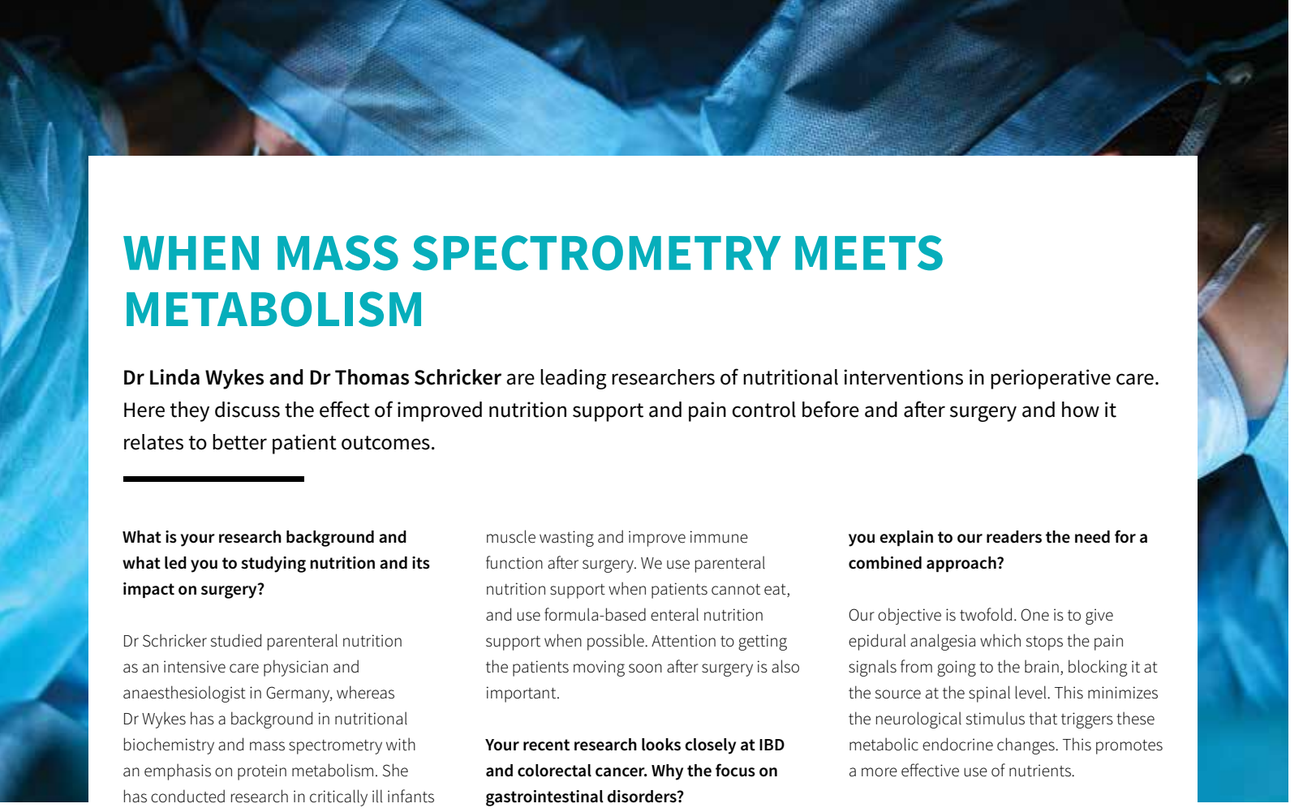

WHEN MASS SPECTROMETRY MEETS METABOLISM

Dr Linda Wykes and Dr Thomas Schricker are leading researchers of nutritional interventions in perioperative care. Here they discuss the effect of improved nutrition support and pain control before and after surgery and how it relates to better patient outcomes.

What is your research background and what led you to studying nutrition and its impact on surgery?

Dr Schricker studied parenteral nutrition as an intensive care physician and anaesthesiologist in Germany, whereas Dr Wykes has a background in nutritional biochemistry and mass spectrometry with an emphasis on protein metabolism. She has conducted research in critically ill infants and later developed a piglet model to study amino acid metabolism in intravenously fed infants. Dr Schricker's clinical expertise specializing in glucose and Dr Wykes' mass spectrometry expertise specialising in protein make for a complementary team.

Tell us more about NASTY

We study Nutrition and Anaesthesia during Surgery as a Team including endocrinologY. We've been working together as a team for 18 years.

We are looking at the nutrition of elective surgery patients going through major surgical procedures with temporary oral food intolerances. A lot of our research has been with patients who need to have surgery for colorectal cancer. Typically patients undergoing intestinal surgery are fasted for several days. This is a problem because the stress of surgery promotes a breakdown of body protein.

How does improved nutrition support affect a patient's surgical outcomes? What nutritional interventions are utilised in your studies?

Our objective is to speed patient recovery by maximizing organ function during invasive procedures like cardiac surgery. Providing effective nutrition support will also decrease muscle wasting and improve immune function after surgery. We use parenteral nutrition support when patients cannot eat, and use formula-based enteral nutrition support when possible. Attention to getting the patients moving soon after surgery is also important.

Your recent research looks closely at IBD and colorectal cancer. Why the focus on gastrointestinal disorders?

We use the anabolic approach of intensive insulin therapy in cardiac surgery which is a very invasive and stressful surgery. In patients with colorectal cancer, we use the anti-catabolic approach of epidural analgesia to decrease cytokines and counter regulatory hormones like cortisol. Against that background, individualized nutrition support gives glucose and amino acids that can be used effectively by the body particularly when perioperative fasting is avoided.

There are several parallels between the metabolic stress of surgery and the inflammation of inflammatory bowel disease. Both are associated with a decrease in dietary intake (e.g. food avoidance due to the pain of IBD) and increased protein and energy requirements due to the cytokines and counter regulatory hormones like cortisol. We are studying a piglet model of IBD so that we can use precisely defined diets to study the separate effects of malnutrition and inflammation. We also use stable isotope tracer infusions in the piglets to study protein synthesis. Using stable isotopes in the piglets means that we can develop new techniques and novel therapeutic interventions and then transfer them to patient studies.

Your study involves elements of pain control and nutritional intervention. Can you explain to our readers the need for a combined approach?

Our objective is twofold. One is to give epidural analgesia which stops the pain signals from going to the brain, blocking it at the source at the spinal level. This minimizes the neurological stimulus that triggers these metabolic endocrine changes. This promotes a more effective use of nutrients.

The second half of the approach is to give nutrition support, to give intravenous glucose and amino acids that can be more effectively used to synthesize protein, in the less catabolic environment enabled by the epidural analgesia. More recently, we found that avoiding perioperative fasting by initiating nutrition support even before surgery means that that patients can synthesize more protein even days after surgery. We've been working on finding the best dosing of amino acids that we're giving. Is it better to give a higher amount of proteins? Or is that too much? The patient may not be able to use the extra amount of amino acids.

We have three methods: epidural analgesia, avoidance of perioperative fasting and a strong endocrine intervention of perioperative intensive insulin therapy.

How do you plan to take this research forward in the future?

Taking this research forward in the future, we're looking at outcome studies to find the best diet for a surgical patient and try to find out if that really makes a difference short term and long term. We would also like to use our precise metabolic kinetic outcomes to see if we can find a biomarker to screen which patients would benefit most from intensive individualized nutrition support.

ENHANCING NUTRITION FOR OPTIMAL OUTCOMES

Using novel approaches such as stable isotope tracers and insulin clamp studies, researchers at McGill University seek to investigate how a combination of nutrition and analgesia can promote anabolism and reduce malnutrition in patients undergoing surgery

The Dangers of Perioperative Malnutrition

Surgery is an essential part of medicine, saving and improving the lives of countless individuals every year. In spite of the overall benefits, surgery also has associated risks, one of which is malnutrition. But how are the two connected?

Firstly, surgery induces a stress response in the body which, if left uncontrolled, can lead to delayed wound healing, loss of muscle mass and poor immune function. This catabolic response may be worsened by excessive fasting and pain. At particular risk of malnutrition are infants, children and the elderly.

Secondly, lengthy fasting can aggravate tissue breakdown. Although academic research advises fasting for six to eight hours before surgery to prevent aspiration, fasting from midnight is still typical practice in many hospitals. In cases of colorectal surgery, fasting may last up to 40 hours due to the bowel preparation involved in the procedure. Such extensive fasting may severely deplete glycogen stores, leading to amino acids being utilised for energy rather than for tissue repair. This may be further exacerbated in those living with type 2 diabetes due to their pre-existing insulin resistance. Excessive nutrition is also risky as morbidity and mortality can increase if blood glucose levels are too high after surgery.

An increasing amount of research is pointing to nutritional interventions to improve recovery times and patient outcomes. Both pre-operative and post-operative nutritional support can reduce the catabolic stress response associated with surgery. Animal studies have demonstrated that this response is greatly improved if animals are fed rather than fasting before trauma. Avoidance of fasting, with particular attention given to the amount and type of protein, can reduce complications, shorten hospital stay and improve overall nutritional status.

When Research Gets NASTY

The NASTY (Nutrition, Anesthesiology and Surgery Team including EndocrinologY) team at McGill University are at the forefront of this research, combining expertise in anaesthesia, kinesiology and mass spectrometry to develop solutions to excess catabolism in surgical patients, as well as in patients with cancer and inflammatory disorders, such as inflammatory bowel disease. In their unique research, Dr Linda Wykes and Dr Thomas Schricker utilise multiple stable isotope tracers to investigate the effect of pain relief and nutrition support on perioperative malnutrition.

The overall aim of nutrition support is to reduce gluconeogenesis – a process in which amino acids are used to make glucose rather than protein synthesis. In order to promote whole body anabolic gains, interventions incorporate a combination of amino acids and glucose. Due to the biochemical link between protein and glucose, nutritional balance depends on an adequate intake of both macronutrients as a deficiency of energy impedes on the benefits of increased amino acids, and vice versa. When asked if a one-size-fits-all solution was possible, the team stressed the importance of personalised intravenous and oral nutrition support regimens: "We tailor the calories of the individual patient's measured resting energy expenditure as assessed by

calorimetry, and then give protein as a percentage of energy needs".

The studies also involve mechanisms of pain control. Epidural anaesthetic is given to offset the catabolic response to surgery. The stress hormones released by the endocrine system stimulate insulin resistance and protein breakdown. However, if pain signals never reach the brain, the flow of stress hormones is decreased and the body is able to make better use of nutrients.

Explaining the Role of Insulin

Gluconeogenesis is also aggravated by impaired insulin sensitivity. Intravenous administration of glucose often leads to hyperglycaemia, a state in which blood glucose is too high. This can increase protein breakdown and reduce the positive effect of feeding on wound healing and recovery. Conversely, due to complex interactions between glucose levels and the body during surgery, attempts to strictly maintain blood glucose levels can result in hypoglycaemia, a state in which blood sugar levels are too low. Therefore, the team aims to maintain normoglycaemia, or normal blood sugar levels, in order to stimulate protein synthesis and decrease tissue breakdown, resulting in a neutral protein balance.

In 2004, the team developed the concept of GIN – Glucose and Insulin administration while maintaining Normoglycaemia – as an alternative to insulin sliding scales in controlling blood glucose. GIN utilises a method known as the hyperinsulinemic-normoglycaemic clamp technique which modifies the rate of glucose infusion while the rate of insulin infusion remains the same, allowing for the maintenance of normoglycaemia. In 2011, the team developed a computer program which allowed newly introduced operators to use GIN safely in surgical patients.

Speeding Recovery for Most Vulnerable Patients

In their 2013 study, Wykes and Schricker aimed to determine if a patient's preoperative catabolic state influenced the anabolic effect of nutritional support. Glucose and amino acid infusions were administered to 17 patients over 72 hours, beginning in the 24 hours before surgery until 48 hours after surgery. The team was then able to measure and analyse the outcomes of their interventions using stable isotope tracer techniques. This involved intravenously infusing amino acids and glucose with stable non-radioactive tags. These tracers mix with other amino acids and glucose and are taken up by the body's tissues. Thus, it was possible to ascertain the rates of glucose production and protein synthesis using mass spectrometry to analyse blood, breath and tissue samples. The results showed a significant association between preoperative catabolism, patient age and the anabolic effect of hypocaloric nutrition.

In a 2008 study, the team explored if the avoidance of preoperative fasting reduced protein breakdown after surgery by examining the effect of intravenous nutrition on 22 patients undergoing colorectal cancer surgery. Patients were randomly assigned to a glucose or amino acids group and the effects were analysed using mass spectrometry. The study found that preoperative hypocaloric feeding significantly reduced endogenous protein breakdown and amino acid oxidation, resulting in a positive protein balance after surgery. As well as showing a net gain in body protein, surgical patients undergoing active nutrition interventions feel better and are discharged earlier.

The team also carried out a study on patients undergoing open heart surgery in 2015. As well as receiving intensive insulin therapy, patients were given amino acid infusions at either 0, 20 or 35 per cent of their resting energy expenditure. Thus, the team were able to determine that the high dose amino acid infusion prevented a decrease in the body's postoperative amino acid concentration, whereas the moderate dose did not. This suggests that these high dose infusions may increase the availability of amino acids for protein synthesis in stressed patients.

The potential impact on perioperative practice is significant. By introducing individualised parenteral or intravenous nutrition to fasting patients, nutrition regimens can be given until the beginning of surgery which can improve postoperative recovery massively. So what's next for the NASTY team? As well as refining the best possible techniques for perioperative nutrition support, these researchers are hoping to investigate the short term and long term outcomes of each intervention and develop further means of identifying which individuals would benefit most from dietary interventions.

Meet the researchers

Dr Linda J. Wykes
Director of the School of Dietetics and Human Nutrition at McGill University

Dr Thomas Schricker
Chair, Department of Anesthesia, Faculty of Medicine, McGill University and Chief, Department of Anesthesia, McGill University Health Centre (MUHC)

Dr Linda J. Wykes received her PhD for work at the Hospital for Sick Children studying amino acid metabolism to develop new total parenteral nutrition regimens for neonates. She was awarded a grant by the Canadian Foundation for Innovation Leaders Opportunity Fund to establish the Mass Spectrometry and Molecular Nutrition Laboratory. Her research focuses on how novel nutrition interventions can overcome insulin resistance to promote anabolism in piglet models and patients.

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Dr Thomas Schricker received his PhD from Ulm University, Germany, and is a founding member of the Perioperative Programme at McGill. His research focuses on mechanisms of the body's stress response to surgery and how this can be modified by anaesthetic and anesthetic, analgesic, hormonal and nutritional interventions to reduce perioperative morbidity.

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TRAINING THE NEXT GENERATION OF BIOSCIENTISTS

In this edition of Scientia, we have highlighted the latest in cutting-edge bioscience research, from creating new technologies to test the potency of stem cells, through investigating the role of fat tissue in metabolism to developing novel approaches to treat ADHD. So, what does the future hold for bioscience research? Who will continue this legacy of continually improving our healthcare systems, and increasing our understanding of life on earth? To close this edition of Scientia, we are featuring two very special articles, introducing university training programs designed to prepare the next generation of bioscientists for careers in research.

First of all, we highlight two Ontario-based research training programmes known as Cancer Research and Technology Transfer (CaRTT) and Partners in Experiential Learning (PEL). Arising from progressive Canadian policies that aim to advance research training and education, these two programs seek to produce top-notch scientists and experts. Under the leadership of Professor Jim Koropatnick, CaRTT at Western University offers training in a vast range of sciences for graduate students and postdoctoral fellows alike. As part of a team effort with Rodger Dusky, Professor Koropatnick also creates opportunities for high school students to participate in health research through the PEL programme.

Meanwhile in the US, Drs Graciela Unguez, Ida Chow and Karen Bennett of the Society of Developmental Biology are working to address the lack of diversity in STEM research, through the Choose Development! program. The programme has already begun to increase the number of students from under-represented minorities and under-served populations that enter graduate programs and then progress to independent research positions. In addition to helping these students achieve their full potential, Choose Development! is helping to increase equality whilst bringing talented individuals into the STEM workforce.

PAVING THE WAY FOR THE FUTURE THROUGH INNOVATIVE TRAINING AND RESEARCH

Determined to have a commanding presence on the scientific global stage, Canada has developed crucial plans to generate high yield research opportunities. Two research training programmes known as Cancer Research and Technology Transfer (CaRTT) and Partners in Experiential Learning (PEL) seek to produce top-notch scientists and experts.

The history of the CaRTT and PEL

The PEL programme, along with its parent programme CaRTT, emerged from deliberate and progressive Canadian policies that have aimed to advance research training and education. As part of strengthening the health research community, the Canadian Institutes of Health Research (CIHR) developed the Strategic Training Initiatives in Health Research (STIHR) in 2002. This initiative seeks to support multidisciplinary research collaboration as well as to incorporate knowledge translation, ethical conduct, and professional skills in training programmes.

Under the leadership of Professor Jim Koropatnick, CaRTT (which originated as a STIHR program) strives to recruit and motivate profoundly qualified individuals to pursue superior training. Joined by Rodger Dusky, Professor Koropatnick also creates opportunities for high school students to participate in health research through the PEL programme.

Emergence of new curricula

In 2009, the National Sciences and Engineering Research Council of Canada (NSERC) established the Collaborative Research and Training Experience (CREATE) programme that initiates strategic training programmes (STPs) in STIHR-related areas such as cancer biology and mental health, and CREATE-related topics that include clean combustion engines. These promote multidisciplinary research opportunities, particularly at the interdisciplinary frontiers among natural sciences, engineering, medicine, social sciences, and humanities. The strategy is to achieve greater impact for trainees by combining the strengths of disciplines that are too-often traditionally separated. STPs are flexibly designed to accept and integrate new educational and innovative discoveries into the training curricula of universities. Some of the methods used to accomplish these goals include interactive online courses and workshops.

The approach to multidisciplinary research has evolved considerably as advocates call for the incorporation of new educational strategies. The traditional health sciences curriculum for graduate and postgraduate education requires the trainee to focus on a particular topic while under the guidance of an expert mentor. However, educators have updated and expanded the framework of this training model. For example, CaRTT trains students beyond one concentration area as it merges translational and multidisciplinary science. It also exposes the trainees to a diverse spectrum of career paths.

What does CaRTT provide?

As a newer paradigm, CaRTT at Western University offers training in a vast range of sciences for graduate students and postdoctoral fellows. The faculty consists of clinicians and scientists from all disciplines who study the methods of diagnosis, treatment, management, and prevention of cancer and other diseases.

The programme takes on 35 graduate students, postdoctoral Fellows, clinical residents and Fellows in translational cancer research each year. The programme director, Professor Koropatnick, describes its three main aims: ‘The purpose of CaRTT is to (a) add disease-based training to students’ university departmental training, which is primarily based on various disciplines (microbiology, immunology, physiology, pharmacology, biochemistry, etc.) rather than disease, (b) bring together clinical and basic researcher trainees from multiple disciplines to collaboratively pursue translational cancer research goals, focused on making a difference to the lives of cancer patients and not only the achievement of academic goals, and (c) train cancer researchers in the art and science of interacting with the private sector to support cancer research through the “valley of death” – the phase of biomedical research immediately following public sector funding

‘A large number of students attending high schools in Canada have the motivation, energy, focus, and ability to become the next generation of leaders in science, technology, engineering, mathematics, and medicine’

of lab-based research in universities and research institutes and before major clinical trials of new ways to prevent, diagnose, and treat disease.’

One of the many ways that CaRTT is fulfilling these goals is by designing a ‘meet-and-interact’ platform that facilitates the sharing of information among laboratories, clinical settings, the private sector, and the community. This programme fosters an environment that unites trainees and mentors as they present their research together and learn about worldwide scientific discoveries. Furthermore, all trainees join a community of research scholars that are connected not only to those in similar areas of study but also to scientists in other fields.

The curriculum also includes remarkable events such as the Annual Cancer Research and Education Day, an Annual Retreat, and monthly seminars in Translational Cancer Research. Other components of the programme include required courses in the sciences and translational cancer research, which equip the trainees with a solid foundation of knowledge and the critical thinking skills to advance their research outcomes. The research programmes at Western University include the following: Translational Breast Cancer Research Unit, Translational Head and Neck Cancer Research Group, Translational Prostate Cancer Research Group, Translational Ovarian Cancer Research Group, Metastasis Translational Cancer Research Team, and Translational Gastrointestinal Cancer Research Team. All of these are associated with cutting edge investigations and collaborations.

The main departments at the Western University are the Cancer Research Laboratory Program, Cancer Research Laboratory Program, and Physics and Clinical Imaging. Furthermore, the university’s affiliation with the Schulich School of Medicine & Dentistry also contributes to a wide array of research opportunities in diabetes, Amyotrophic Lateral Sclerosis (ALS), rosacea, and other areas as well.
The CaRTT programme is determined to nourish its trainees with a comprehensive depth of knowledge through several ways. As a key element of the programme, CaRTT has teamed with the Richard Ivey School of Business at Western University (ranked by Bloomberg Businessweek in 2014 and 2015 as the best international business school outside the United States and the top school in Canada) to present its students with a business course to develop their skill at teaming curiosity-driven, publicly-supported basic research with private sector-supported applied research – an essential step in allowing scientific discovery to achieve impact in reducing the burden of disease. Additionally, it stresses the building of professional skills in communication, grant writing, and peer review.

PEL: meeting the needs of higher education

Partners in Experiential Learning, or the PEL programme, was established in 2004 to encourage talented high-school students to pursue careers in science, technology, engineering, mathematics, and medicine (STEMM). ‘The purpose of the programme is to fuel the excitement of top-level students in pursuing a career, not only in cancer research specifically, but in a broad range of STEMM disciplines. In addition, the programme makes them knowledgeable about the set of skills they need to successfully pursue a STEMM career’ Professor Koropatnick explains. 60 students per year are recruited to the programme, who undertake mentor guided projects that cover STEMM topics as outlined by the Ontario curriculum. The students are involved in experimentation, data analysis and interpretation, grant writing, and even publication authorship.

The mission of the PEL programme serves to support students in their quest of discovery and knowledge translation in the STEMM subjects by pairing them with mentors. The students are encouraged to pursue academia and career paths that serve the benefit of Canadians. In this way the programme directly addresses the looming shortage of highly-qualified personnel in STEMM careers in Canada, and helps Canadian youth to

reach their full potential. Additionally, it seeks to provide students with connections that they can maintain throughout their future and career training.

The PEL programme inspires its students to engage in conferences, seminars and research days. For example, CaRTT along with the Translational Breast Cancer Research Unit host a joint monthly Translational Seminar Series in which students are invited to attend lectures presented by world leading scientists. Furthermore, students participate in The Annual CaRTT and Department of Oncology Research and Education Day, and meet annually with winners of the Canada International Gairdner Awards (traditionally considered a precursor to winning a Nobel Prize in Medicine).

Designated STEMM institutions include the Lawson Health Research Institute, St. Joseph’s Health Care London, the London Health Sciences Centre, the Robarts Research Institute, and the London Health Sciences Centre.

How do these programmes impact the community?

Canada recognises that it needs more researchers in the STEMM disciplines and a greater number of PhD graduates who can contribute their expertise in non-academic settings. In fact, the Conference Board of Canada in 2014 reported that its country is lacking in productivity, growth, and innovation. Among the Organisation for Economic Co-operation and Development (OECD) countries, Canada ranks 21st. Hence, there is a sense of urgency to reduce these deficiencies.

While many complete undergraduate education in STEMM disciplines, unfortunately, a majority do not pursue further training or careers related to STEMM. The reason for these mediocre numbers is explained by the limited number of programmes dedicated to STEMM education and career cultivation especially during the early phases of training. Furthermore, bright young students are not receiving adequate exposure to STEMM career paths during high school, which is the crucial period when students decide what their future entails.

However, the PEL programme is very promising as Canadian high school students possess the enthusiasm, motivation, and capability to be the future of STEMM training. Moreover, this programme is one way to minimise the STEMM deficit. Since its launch in 2004, it has graduated almost 500 students. The vast majority of the early PEL scholars have gone on to pursue STEMM career paths.

Who are the mentors?

The researchers involved in mentoring and guiding students and trainees are engaged in a myriad of fundamental investigations ranging from cancer to physics to public health policy. They conduct research in molecular mechanisms and signalling pathways involved in breast cancer, prostate cancer, lung cancer and ovarian cancer, to name a few. Scientists also study the process of metastasis, which is described as the spread of cancer from the original organ to other sites. Other researchers explore mechanisms underlying resistance to cancer treatment, including enhanced cancer cell capacity to repair damage induced by anticancer drugs and radiation and to evade recognition by the immune system, and develop ways to overcome that resistance.

Pharmacology is another interest among particular scientists as some embark upon the design and preparation of new drugs in the treatment of cancer. Also, researchers assess various approaches in cancer therapy such as the use of novel vaccines in the prevention and treatment while others study the enhancement of current and new tumour drugs through gene manipulation. Further laboratories are working on inventing immunotherapeutic drugs for a broad spectrum of diseases as one laboratory, in particular, is eager to develop an anti-inflammatory therapy for spinal cord injuries.

Scientists also study disorders that affect the head and neck, lungs, skin, and other organs. The specific interests include asthma, chronic obstructive pulmonary disease (COPD), type I diabetes mellitus, paediatric pancreatic disease, and organ transplantation. Other research areas concentrate on stem cells, developmental genetics, and even the role of hormones in growth and development of the foetus.

Radiation oncology techniques, nanotechnology and computer modelling of biological systems are all significant fields of research. For example, these studies have led to the invention of photoacoustic imaging – a technique used to detect breast cancer as well as many other diseases. Additionally, physicists are striving to develop new methods that optimise ultrasound, magnetic resonance (MR) and positron emission tomographic (PET) imaging. Others direct their efforts towards synthesising biodegradable polymers that are useful in tissue engineering, medical instruments and drug delivery.

A key goal of CaRTT and PEL is to train future researchers to exploit the ‘merge points’ of disciplines, where different ideas and perspectives meet to create productivity greater than is possible through any isolated discipline.

‘A frustratingly large number of talented, high ability trainees do not continue with postgraduate STEMM training and successful STEMM careers’

The future: educating a new generation

Alumni of both programmes may pursue careers in a diverse range of sectors such as academia, clinical settings or industry. Additionally, they may serve in government appointments to guide and form research policy.

There has been positive feedback from the students and mentors regarding CaRTT and PEL. Also, faculty mentors help with curricular planning as they provide resources and mentorship. CaRTT and PEL are highly committed to the enhancement of the educational and training experience as well as the personal growth of the participants.

The Canadian Academy of Health Sciences (CAHS) challenges academic institutions to promote and advance research for the sake of science, and the benefits provided to both government and industry. Students are encouraged to fulfil their maximum potential and to join the talent pool in the research community. Their future discoveries may lead to economic development and intellectual advancement for the greater good of the country.

Meet the researchers

Professor Jim Koropatnick
London Health Sciences Centre
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Rodger L. Dusky
Partners in Experiential Learning (PEL)
London
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Professor Jim Koropatnick serves as the Director of the Canadian Institutes of Health Research Strategic Training Program in Cancer Research & Technology Transfer, Cancer Research Laboratory Program, the London Regional Cancer Program, and the London Health Sciences Centre. He is also a faculty member at the University of Western Ontario in the Department of Oncology. His research interests focus on the treatment of tumour cells and the identification of genes that lead to therapy resistance. Ultimately, his goal is to optimize treatment through gene manipulation. Professor Koropatnick's laboratory recruits graduate students, research associates, visiting researchers, postdoctoral fellows and summer students.

Rodger Dusky is a retired chemistry, science, and Head of Cooperative Education who served in senior composite and academic secondary schools in Ontario. In 1995, he was awarded the National Prime Minister's Award for Teaching Excellence in Science, Technology and Mathematics. With Professor Koropatnick, he created PEL in 2003 to match promising science co-operative students with mentors in the STEMM research community. He continues as Director of PEL, member of the CaRTT Program Advisory Committee, coordinator of the UWO Schulich School of Medicine and Dentistry Secondary School Gairdner Event, and member of the Ontario Provincial Partnership Council and its Strategic Task Force Committee.

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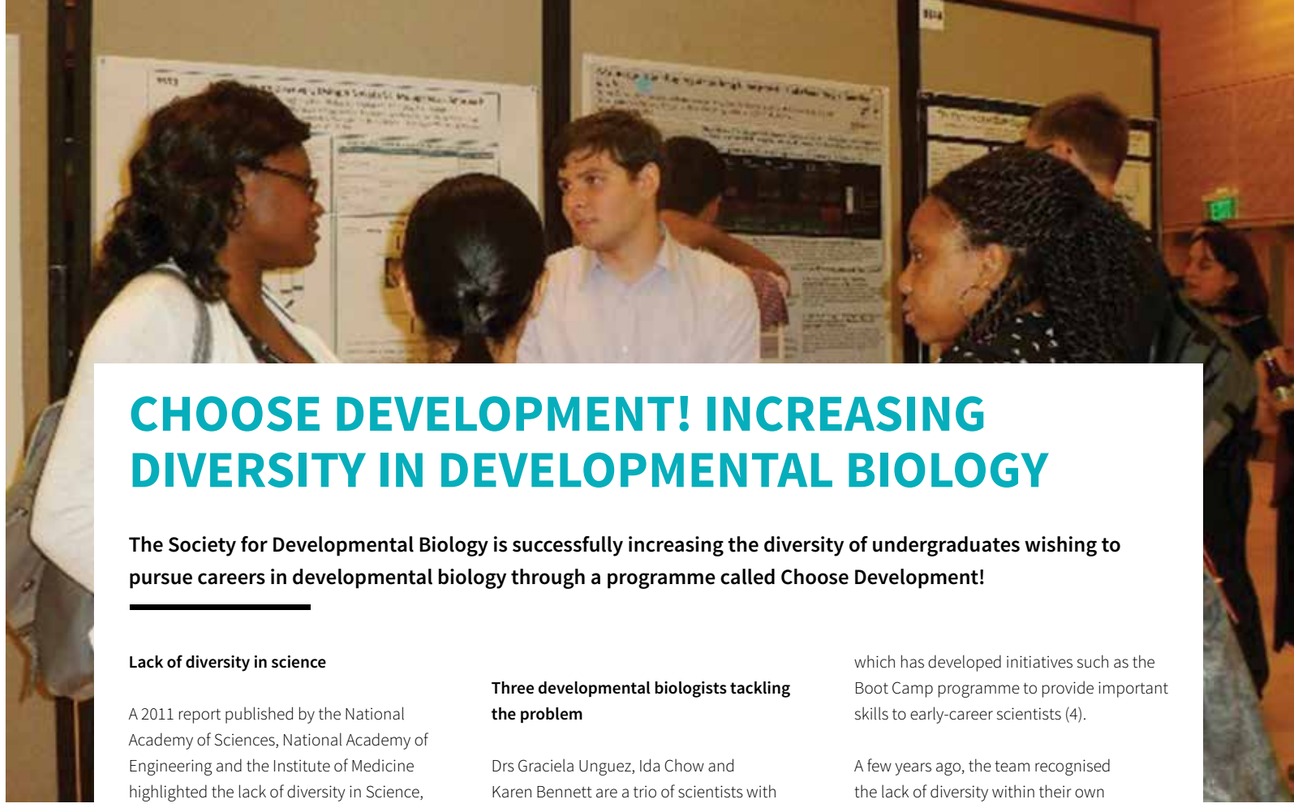

CHOOSE DEVELOPMENT! INCREASING DIVERSITY IN DEVELOPMENTAL BIOLOGY

The Society for Developmental Biology is successfully increasing the diversity of undergraduates wishing to pursue careers in developmental biology through a programme called Choose Development!

Lack of diversity in science

A 2011 report published by the National Academy of Sciences, National Academy of Engineering and the Institute of Medicine highlighted the lack of diversity in Science, Technology, Engineering and Maths (STEM) disciplines. Under-represented minorities (URMs), including African-Americans, Hispanics/Latinos and Native Americans, hold only about 6% of STEM-orientated jobs, despite making up 29% of the US population (1). One of the major issues is the lack of URMs completing university education – URMs make up 33% of all secondary graduates, yet this drops to 26% when considering students enrolled in undergraduate courses, and worse, 18% when considering students that complete STEM-oriented degrees.

One of the main reasons cited for the low number of STEM graduates is that URM students fail to see themselves as successful scientists, due to insufficient mentoring, peer support, and a lack of role models and support from their community (2). This highlights a self-defeating cycle, whereby the low percentage of URMs in the STEM workforce discourages further URMs from studying STEM subjects. However, URMs represent a massive untapped talent pool for future scientists. In addition, multiple studies have shown that increased employee diversity is associated with increased creativity and productivity (3). The demographics also predict a significant increase in URMs in the US population in the near future.

Three developmental biologists tackling the problem

Drs Graciela Unguez, Ida Chow and Karen Bennett are a trio of scientists with backgrounds in developmental biology. When asked what drew each of them to the field of development, Dr Unguez responds that 'the well-known capability of all organisms – plant and animals – to modify their cells, tissues and organs in order to better adapt to changes in environmental conditions has been, to me, the most exciting developmental process of a fully "differentiated organism"'. Similarly, the big question that drew Ida Chow to developmental biology was 'how does one cell, the fertilized egg of all organisms differentiate and develop into such complex and beautiful structures?'. Dr Bennett tells us that the application of developmental biology to medical therapy is what drew her to the field: 'I was attracted by and am still extraordinarily proud of the exciting future of our field in terms of stem cell research, disease remediation, and understanding the process of life'.

In addition to their research contribution to the field, the trio also has a strong background in outreach, owing to their involvement with the Society for Developmental Biology (SDB). Dr Chow has been involved in multiple National Science Foundation (NSF) funded collaborations between the SDB and the Latin American Society for Developmental Biology. All three are also part of the SDB Professional Development and Education Committee, which has developed initiatives such as the Boot Camp programme to provide important skills to early-career scientists (4).

A few years ago, the team recognised the lack of diversity within their own organisation (as in many other US science organisations) – URMs made up only about 10% of the Society, compared to 29% of the US population. They also knew that typical programmes targeted at URMs in the past have been slow in impacting the demographics (despite high initial participation) because these opportunities largely depend on very short-term exposure to the sciences. As Dr Chow describes: 'It has been known and documented that students from currently under-represented minority and under-served populations, students with disabilities (visible and invisible) and from economically disadvantaged communities need continued mentoring and for longer periods, to encourage and cultivate their interest for science and research (2). Unfortunately, most of the existing programs for these students are one-time deals, raising their interest but not providing continuity to sustain and increase their participation in science'.

These facts spurred the trio to develop a long-lasting approach to increasing diversity within the Society. Together, the team developed a programme called 'Choose Development!', and secured $285,000 of NSF funding under the BIO-Integrative Organismal Systems' 'Broadening Participation' initiative (5). 'The two main goals of the programme are to increase the number of URM undergraduates and students with

‘The Choose Development! programme represents a unique approach to recruiting and retaining underrepresented minorities into science careers’

disabilities entering graduate programs in developmental biology and progressing to independent positions, and to increase and retain the number of SDB members from the URM and persons with disabilities populations by at least 25% of the current figures (72 of 681 survey respondents) by the end of this Choose Development project funding period’, Dr Chow tells Scientia.

Bringing innovation to URM-targeted programmes

The Choose Development! programme brings a fresh approach to increasing URM representation in science. The programme scouts for talented URMs in their undergraduate studies, and brings them under the wing of enthusiastic mentors from the SDB – talented scientists who can offer inspiring environments to learn about science and developmental biology. Moreover, to retain them in the field of developmental biology over the long term, SDB offers Choose Development Fellows the support of a community throughout their university degrees and beyond.

Once chosen, programme participants (Fellows) do a summer research project with their mentors. The next year they attend the SDB annual meeting to present their work, develop their professional skills, and build relationships with developmental biologists at all stages of their careers. SDB Fellows are encouraged to do additional summer internships throughout their undergraduate studies. Participants have had the opportunity to work at a number of institutions across the US, including, for example, San Francisco State University, The University of Puerto Rico, Princeton University, the University of Utah, New York University and Memorial Sloan Kettering Cancer Centre. In addition, a fortuitous partnership between SDB and the historic Embryology Course at the Marine Biological Laboratory in Woods Hole, Massachusetts has enabled two Fellows per year to spend one week at the course expanding their knowledge of developmental biology experimental techniques and diverse embryonic systems.

The integration of participants into the SDB community gives them a strong identity and sense of belonging within the Society and sets them up for their careers in science. As Dr Bennett describes: ‘SDB Fellows, as our selected students are called, do more than summer lab work; they are recognized and have an identity in our Society and throughout the field of developmental biology. Along with working in the laboratories of successful scientists during several summers, each Fellow is brought to the annual meeting with one of their mentors, is highlighted throughout the meeting with videos of their work, and is received both at an SDB Board reception and at the Awards Banquet. In the past three years our SDB Fellows have bonded with one another throughout the meetings and through social media, and have been extraordinarily successful in being accepted into prestigious graduate programmes’.

The benefits of the programme work in many ways. Not only do the Choose Development! Fellows benefit, but their mentors also gain valuable skills. Along with the mentoring provided by the lab head, the SDB Fellows are also assisted by a designated lab mentor. As Dr Chow describes: ‘Our multilevel mentoring has not only helped the students, but also provided learning and training experience for the laboratory mentors, mostly postdoc fellows who will one day have their own labs and will need to mentor students’. The programme provides mentors with a 2-day workshop, and continual assistance from ‘Master’ mentors. The trio also hope that the programme will help translate science to communities, as their participants are encouraged to perform outreach activities.

Programme results

The Choose Development! programme is completing its third year, and has successfully obtained bridge funding for a further two years. As Dr Unguez notes: ‘students becoming a member of a team to learn science, and being welcomed into an entire scientific society by its members, is an extremely doable accomplishment in building [an inclusive scientific] community’.

The success of the programme is reflected in the results – Fellows showed greater learning across many aspects compared to the average student population (using the Grinnell College SURE III (Survey of Undergraduate Research Experiences) and interviews). Gains were especially noticeable in defining career paths, understanding scientific literature, and oral presentation. One of the most positive effects of the programme was an increased willingness of participants to take part in research again, and this is exemplified by the number of programme participants who are now enrolled in postgraduate programmes (five out of nine who have already graduated), medical school or working in laboratories – an encouraging result for the future of URMs (and the Choose Development! programme) in the SDB.

Throughout these three years, the number of URM trainee members in SDB has increased by about 20%, in part as a result of active promotion of the Choose Development! Programme, and recruiting potential candidates at URM-targeted meetings, in addition to within the SDB membership.

‘Our multilevel mentoring has not only helped students but has also provided learning and training experience for laboratory mentors’

The Future for Choose Development!

The team behind Choose Development! is hoping that SDB members will take the programme forward by providing outreach to their communities, and further encouraging URMs to take part in the programme and STEM subjects. They all agree that their main goals are to sustain this initiative with long-lasting funding, to improve the practices used by incorporating suggestions from the ongoing evaluations, and to establish partnerships with other scientific societies, universities and research institutions.

It is the hope of this team that the programme will inspire others and provide the basic groundwork for diverse scientific societies and organisations to implement similar programmes inspiring URMs. As Dr Unguez puts it: ‘Choose Development is a training programme that addresses all stakeholders in a scientific society and informs all members of the overdue necessity to widen the inclusion of young trainees into the biological sciences. The programme does not function in isolation, it reaches out to all members of our scientific society to participate, open their labs for summer training and open opportunities for graduate students and post-doctoral fellows to also include useful mentoring skills into their career toolbox’.

Indeed, similar pushes for diversity in science are being funded across the world – the UK has a similar focus in their ‘Leading the way: Increasing Diversity in the Scientific Workforce’ programme. By increasing the number of URMs completing science degrees, this talented team hopes to eventually bring equality to the STEM workforce, and uncover the massive untapped potential in minority communities. The team knows that the best way to do this is by providing a supportive community to URMs, and has facilitated its connections with the SDB to help make their programme successful and serve as a source of inspiration for others in science.

Interested in contributing or taking part?

The Choose Development! programme is open to URM undergraduates or those with disabilities, who are US and Canadian nationals or permanents residents. Preference will be given to undergraduates in their first or second years of study. If selected, candidates may be required to travel within the US (assistance with travel and accommodation is provided).

Those wishing to mentor as part of the Choose Development! Programme must be SDB members who hold research grants, and can provide sufficient capacity and training for a student, including a lab mentor (either postdoc, lab manager, or advanced graduate student). People interested in partnering with SDB or becoming a Choose Development! mentor or fellow are invited to contact Dr Chow.

Meet the coordinators

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The Choose Development! programme was created by a collaboration between three key members of the Society for Developmental Biology: Dr Graciela Unguez, Dr Ida Chow and Dr Karen Bennett. Dr Graciela Unguez is the Principal Investigator and Professor of Biology at the New Mexico State University, with a subject focus on the development and regeneration of electric fish tissues. Dr Ida Chow is the Co-Principal Investigator of the project, Executive Officer of the Society for Developmental Biology, and a Fellow of the American Association for the Advancement of Science. Her previous research focused on the formation of neuromuscular junctions in the frog. Dr Karen Bennett is the Program Coordinator and an Emerita Professor from the University of Missouri, with a subject focus in nematode developmental biology. In addition to their research, they coordinate the Choose Development! programme, and are also involved in the Society's Professional Development and Education Committee.

W: http://www.sdbonline.org/

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SDB Boot Camp for New Faculty. http://sdbonline.org/boot_camp

SDB Choose Development! http://www.sdbonline.org/choose_development

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