Developing heart stem cell expertise
Report on the ICMR visit to San Diego State University Heart Institute

Scientists of the future
Students share their experiences of the undergraduate vacation studentships

In conversation ...
with Dr Alister McNeish, lecturer in Pharmacology

Research highlights
Eating for your genes
How abnormal platelet function leads to heart attacks and strokes
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Welcome to the first edition of Highlights. Within this publication we aim to highlight some of the Institute for Cardiovascular & Metabolic Research (ICMR) activities that over the past year have contributed to its success.

Since the formation of the ICMR in 2009, we have enjoyed growing success. Our membership has increased by over 50%. We have successfully recruited eminent scientists, including professors, lecturers and postdoctoral fellows. Research training has expanded and broadened, with increasing numbers of PhD studentships and research development opportunities offered by the ICMR award schemes. Our increasing size and expertise has enabled some exciting new possibilities and research opportunities, several of which underpin major advances in cardiovascular and metabolic research. This has resulted in prominent publications, considerable grant income and significant expansion of the ICMR facilities.

The first phase of a planned £2.5 million expansion of ICMR facilities commenced with the official opening of the new vascular suite in the Department of Food and Nutritional Sciences in 2011. These facilities offer a dedicated suite for the determination of human vascular function using cutting-edge technology. Funds from the Garfield-Weston Foundation and the Charles Wolfson Charitable Trust were instrumental in the provision of these facilities, and to whom we are indebted, allowing essential equipment and nursing provision to be secured. The second phase of the ICMR expansion has begun with the foundation of new ICMR imaging facilities that will impact on all aspects of our work. Some equipment has already been acquired and installed, and we plan to expand further to include the most advanced fluorescence confocal microscopy technologies, flow cytometry, ultrasound and whole body imaging. This venture will also involve the consolidation of a number of collaborative groups in a single location and the refurbishment of the required laboratory space. This will enable ICMR research to continue to grow at the forefront of scientific developments, and would not be possible without the exceptional generosity of individual donors to whom we are very grateful.

With infrastructure grants submitted and in preparation, it is hoped that funding to support the remaining aspects of this project will be in place for its completion in the next few months.

ICMR research incorporates a large number of international and cross-disciplinary collaborations. Formal links are under development between the ICMR and the University of Delaware Cardiovascular Research Center and the San Diego State University Heart Institute. In addition, an exchange programme for undergraduate and postgraduate students between ICMR and the two American universities is at an advanced stage and is due to commence in the summer of 2012.

We hope that you enjoy this first edition of Highlights featuring the research and scientific developments within the ICMR.
News

Breakthrough: a new drug target to combat thrombosis

Professor Jon Gibbins, Director of the ICMR, and his group have recently discovered a protein in blood platelets, that regulates normal and pathological blood clotting.

Studying the activity and function of this protein in platelets will lead to a better understanding of how heart attacks and strokes may be prevented. This breakthrough was covered by the media around the world and was also featured on the BBC website health section. For further details please see Research Highlights on page 7.

Making medicine from bacteria

British Heart Foundation (BHF) funded research from Dr Simon Clarke’s laboratory was highlighted as an example of innovative research in the BHF Mending Broken Hearts campaign in the national press, focusing on clues from nature that may change the way that heart attacks and strokes are prevented or treated. Further details of this study can be found in Research Highlights on page 8.

Blueberry research makes prime time viewing

Professor Jeremy Spencer recently appeared on the Channel 4 programme, The Food Hospital where he described the cardiovascular benefits of flavonoid-rich foods. During the programme he and his research team demonstrated the beneficial effects of blueberry on the elasticity of the blood vessels, an indicator of the health of the coronary arteries. The programme was broadcast on 2 November 2011.

New editorial roles for ICMR academics

Two new appointments broaden the ICMR’s role in the publication of high-quality science in a wide range of notable journals that cover the spectrum of cardiovascular and metabolic research disciplines.

In March, Alister McNeish was appointed to the Editorial Board of the British Journal of Pharmacology.

Professor Jeremy Spencer has been appointed as Editor-In-Chief of Nutrition and Aging, published by IOS Press. Nutrition and Aging is an international journal directed to the study of the effects of diets and dietary components on aging and age-related diseases. It is particularly concerned with experimental results emanating from biochemical and pharmacological studies, cardiovascular medicine, neuroscience, and nutritional sciences that demonstrate the effects of dietary constituents on the adverse effects of aging.

Gold award for ICMR member

Professor Christine Williams, the University’s Pro-Vice-Chancellor for Research and Innovation has been awarded the prestigious Nutrition Society Gold Medal for her work in shaping and directing the Society.

As a former President of the Society, Professor Williams was one of seven members honoured with the Gold Medal at a ceremony to celebrate the 70th anniversary of the Society at the Royal College of Physicians in London on the 7 December. A spokeswoman for the Society said: ‘The Gold Medal Award has only been presented once before in the history of the Society and it was a great pleasure to be able to honour our most esteemed members in our 70th anniversary year. All our Gold Medal holders have made a huge contribution to advance the scientific study of nutrition and its application to the maintenance of human and animal health’.

A new vascular function suite

On 15 March 2011, the new vascular suite in the Department of Food and Nutritional Sciences was officially opened. The two rooms were named the Williams Suite, in honour of Professor Christine Williams, the first Hugh Sinclair Professor, and the Garfield Weston Suite, after the trust that provided substantial funding for the refurbishment of the rooms, and purchase of much needed equipment. The new facilities have been used extensively since their completion and have increased substantially the capacity of the Clinical Unit.

This has facilitated grant success including funds from the Charles Wolfson Charitable Trust for the employment of two research nurses to assist in the running of ICMR studies in the Unit.

Volunteers needed for studies conducted at the ICMR

For more information about participating in dietary and other human intervention studies aimed at reducing the risk of heart disease, please contact:

Mrs Jan Luff | Tel: 0118 387 7771 | Email: j.e.luff@reading.ac.uk
In conversation ... 
with Dr Alister McNeish
Lecturer in Pharmacology

Dr Alister McNeish was recently appointed as a lecturer in Pharmacology in the School of Chemistry, Food & Pharmacy and is the newest member of the ICMR. Alister has recently been awarded a three-year project grant from the British Heart Foundation (BHF) to study the way in which different cells within blood vessels communicate. In this recent interview he tells us about his background, research and plans for the future.

Dr Alister McNeish

Congratulations on getting the new BHF grant!

What will you be studying?

I entered cardiovascular research, having previously studied the blood vessels in the eye, as it became apparent to me that they had many similar properties to the blood vessels supplying the brain (cerebral blood vessels). One of the properties that they share is that the endothelial cells that line the inner surface of arteries can electrochemically ‘communicate’ with the smooth muscle cells that constitute the wall of the artery. These electrical signals cause arteries to dilate and thus increase blood flow. Indeed similar mechanisms occur in small arteries in other parts of the body and are critical for regulating blood pressure. The BHF award will give me the opportunity to understand how to modulate these signals. This will not only give information about how blood vessels normally function but also how we might be able to prevent cardiovascular diseases by manipulating these mechanisms. I also have several collaborations looking at other aspects of vascular signalling that may be important in the regulation of blood pressure and prevention of migraines.

Where did you study and train?

I studied Pharmacology as an undergraduate at the University of Edinburgh, where I was given a fantastic opportunity to train for six months in the laboratories of the late Sir James Black, an eminent pharmacologist and Nobel Prize winner. I think this gave me the best training any pharmacologist is ever likely to receive. I undertook a PhD at Glasgow University under the supervision of Professor William Martin, an instrumental researcher in the discovery and mechanisms of nitric oxide signalling and was so encouraging. My postdoctoral training was at the University of Bath under the supervision of Professor Christopher Garland, a world expert on electrical signalling in the vascular system. This work led to the award of a fellowship from the BHF which enabled me to become an independent researcher.

How did your interest in cardiovascular research come about?

I have to admit I was a bit of a latecomer to cardiovascular research and there was an element of serendipity. Throughout my undergraduate degree I studied pharmacology, focusing on neuropharmacology. I began a PhD looking into the function of the eye and how intra-ocular pressure increases could cause damage to the retina. The process of formation of intra-ocular fluid relies heavily on the delivery of fluid via the vascular supply to the eye. I was fascinated by this process and some interesting findings in the ocular vascular system lead to my PhD being solely focused on the arterial blood supply to the eye.

What has your research shown so far?

My PhD research showed that an antioxidant vitamin C could actually impair some aspects of vascular communication, which may go some way to explain why the large-scale trials of vitamin C supplementation on cardiovascular health and function failed. More recently my research has shown that a signalling molecule, nitric oxide, can ‘protect’ electrical communication pathways in cerebral arteries. Cerebrovascular diseases such as stroke are associated with reduced electrical and nitric oxide signalling. I have recently discovered mechanisms that recover electrical communication and vascular relaxation in cerebral arteries – even if nitric oxide signalling is impaired. These mechanisms may have the potential to be exploited clinically to treat cerebro and cardiovascular disease; they may also explain some of the protective effects of the commonly prescribed drugs, statins, on cardiovascular health.
Why did you come to Reading?
Towards the end of my fellowship in Bath, I started looking forward to a fresh challenge and change in research environment. After visiting the University of Reading and meeting members of the School of Pharmacy and members of the relatively new ICMR, my mind was made up. Current research being carried out in the ICMR complemented my own, and the new facilities available at Reading combined to make a very attractive package.

How does your work fit in with other research in the ICMR and the research priorities of the University as a whole?
Cardiovascular diseases have some common risk factors and are often multifactorial, meaning problems in the vascular system may precipitate or exacerbate these diseases. Similarly, cardiac and metabolic diseases, as well as nutrition, have a major impact on vascular function. The opportunity for collaborative research between a variety of different research groups at Reading is tremendous.

What qualities do you believe a successful researcher has to have?
I think you need to have passion for and actively enjoy research as well as a desire to find out new information. Throw into this mix a modicum of tenacity and stubbornness combined with perseverance and you have a research scientist. Conversely, I think you also need to be able to recognise when you can’t do something and use the information and experience you have gained to improve and build your research.

What are the main challenges you face regarding your research?
I see the major challenge being identifying new exciting areas of research that not only complement my own skills and those of my collaborators, but which will also have clear benefits to society. Unfortunately, in the current economic climate funding is becoming harder to get. This makes research more competitive, which, in a way is good, as it encourages us to consider our future research carefully and improve research standards; however, it may also favour established researchers who already have the resources to undertake new research more competitively than a new researcher.

What do you like and dislike most about your work?
I love finding new things out, talking to new and interesting people who may do quite different research. I also enjoy teaching and contributing towards the skills and knowledge of the next generation of scientists. I have to admit my passion is being at the bench and I am not a huge fan of ‘administration’. Being an office for an overly long period of time is not my idea of fun!

What have been your proudest achievements?
That’s difficult; I think the award of my BHF fellowship and being awarded a prize for being one of the most promising young pharmacologists in Britain (2010 Bill Bowman travelling lectureship from the British Pharmacological Society) make me proudest in terms of my career.

Looking ahead, what are your aims for the next few years?
Following the recent award from the BHF, I am looking forward to establishing a research group at Reading. As for other plans, my head is full of ideas for projects I want to investigate and I fully intend to continue building on new collaborations within the ICMR – after all that was the main driver for moving to Reading.
A s public health strategies become increasingly concerned with the prevention of polygenic diseases (those that are affected by a number of different gene variations), such as diabetes and cardiovascular disease, the need for public health interventions aimed at reducing cardiometabolic risk is even greater. Nutritional genomics and genetics will increase our understanding of how the genetic make-up of an individual coordinates their response to food (also referred to as diet–gene interactions), relating this variation to disease states. These tools could revolutionise the way in which we deliver dietary advice for the prevention of these conditions. Personalised nutrition, which uses genetic, phenotypic (modifiable biochemical characteristics such as blood cholesterol) and dietary data to tailor an individual’s diet, may also have a positive impact on motivation to change eating behaviour.

The aims of personalised nutrition are to lower disease risk, improve dietary treatment of disease and to enhance health performance. Food4Me aims to investigate consumer understanding of personalised nutrition in order to identify barriers to its adoption, to explore technologies to support the implementation of personalised nutrition services, and to investigate the impact of gene polymorphisms. The key emphasis will be on gene variations known to influence cardiometabolic disorders such as cardiovascular disease and type 2 diabetes.

**Proof of principle study**

The Food4Me consortium, including the team from the ICMR (Julie Lovegrove, Anna Macready, Rosalind Fallaize) and colleagues from the School of Psychology (Professor Judi Ellis and Dr Laurie Butler), will be conducting a proof of principle human intervention study which will be the first of its kind globally. This study will be designed to mimic an internet-delivered personalised nutrition service, incorporating the use of self-administered, pin-prick blood analysis and buccal swab genetic testing kits. The hypothesis being tested is that participants will achieve increasing levels of success with greater amounts of information concerning the personalisation of their diet. Prior to commencing the study, we will determine subjects’ genotypes (the versions of specific genes that they possess) for genes that are known to affect cardiometabolic risk. In addition, the most suitable phenotyping parameters (such as plasma cholesterol) will be identified, developed and tested using cluster analysis. This information will guide the development of an online dietary assessment tool which will use a set of algorithms for the provision of dietary advice. A recipe advice system will also be formulated for use in this novel study. Researchers at the ICMR and the other collaborating EU partners will recruit 160 volunteers per centre, resulting in a total of 1,280 participants across Europe for the six-month intervention study.

There will be three levels of personalisation:

1. Personalised dietary advice
2. Personalised advice based on diet and phenotype
3. Personalised advice based on diet, phenotype and genotype.

The impact of coaching on dietary change will also be investigated in the study by varying the level of feedback within each level of personalisation. A control group will receive no advice during the project but will receive dietary advice on completion of the project.

**Focus group consumer attitude research**

Members of Food4Me Consortium will also provide insight into consumer perceptions of personalised nutrition. This will facilitate the development of a theoretical model detailing factors (such as trust) that would influence consumer uptake. To achieve this objective, a series of focus groups will be conducted at eight EU centres including Reading. During these focus groups, we aim to elicit information regarding the perceived risks and benefits of personalised nutrition including: the use of genetic information by third parties, potential health benefits for the consumer, and cost implications. Further focus groups will feed directly into the development of the business creation models. In these sessions, potential applications of personalised nutrition in relation to cardiometabolic disease risk will be explored.

**Future directions**

Results from the Food4Me studies will help to guide future research into the reduction of cardiometabolic risk and the prevention and treatment of other nutritionally mediated diseases.
Understanding how abnormal platelet function leads to heart attacks and strokes

The reasons for the occurrence of heart attacks and strokes are complex and usually a consequence of the development of diseased arteries over many years. While the causes of vascular disease are multi-factorial and in some cases controversial, details of how diseased arteries trigger thrombosis (occlusive blood clots that result in rapid damaged to the sensitive tissues of the heart and the brain) is now well established. The culprits are blood cells known as platelets, doing what comes naturally, but in the wrong place.

Platelets are small blood cells that form a front line of defence against injury. They continually survey the integrity of blood vessels and when they encounter blood vessel damage or leak from blood vessels at sites of tissue trauma, they recognise proteins that they would not normally ‘see’ within a blood vessel, and from this they sense that all is not well. They stick to the injury site and then to each other, initially plugging the hole and then helping the blood to clot. Clearly this is vital. If we didn’t have platelets we would leak dangerously following even minor injuries. Platelets, unfortunately cannot tell the difference between tissue trauma and diseased arteries, and particularly the accumulation of fatty plaques (atherosclerosis) in the coronary or carotid arteries. These lesions often possess, or when they rupture release, factors that would normally flag-up tissue injury to a platelet, and as a consequence blood clotting is triggered within a blood vessel.

Suppressing platelet reactivity to injury signals has proven an effective means to prevent thrombosis (this is the basis of the action of low-dose aspirin), but there is a fine balance to be reached between reducing thrombosis and inducing bleeding, and therefore the therapeutic approaches that are currently available are either ineffective or inappropriate for many patients that are at risk of thrombosis.

The focus of the Platelet Biology Group within the ICMR is to understand in finer molecular detail, how platelets see and respond to tissue injury and how they ignore the healthy undamaged or undiseased circulation. This will provide the information required to develop more effective, less dangerous and more refined anti-thrombotic approaches.

Dr Leo Moraes and Michael Spyridon together with a number of colleagues recently made a number of important discoveries that may open up the possibility of new therapies. They discovered a protein in platelets known as LXR. This protein, which is found inside platelets, is known to respond to chemical signals present outside of cells and then orchestrate the regulation of the use of a range of genes that are known to control blood cholesterol levels. LXR has therefore already gained a great deal of attention in the study of cardiovascular disease. Platelets, however, have no nucleus and therefore have no genes to regulate. It seems, that this protein has a double life, and is able to control non-genome related effects in platelets. Drugs have been in development that target this protein, and have previously been tested in mice where they show the ability to reduce cardiovascular disease, although this seems to be unrelated to effects on blood cholesterol levels. This led Leo and Michael to ask the question, do drugs that target LXR (known as ligands) modulate platelet function? Their hunch turned out to be correct.

In a paper published in the journal Blood, they report that LXR ligands reduce platelet reactivity, resulting in less pronounced responses to the factors that platelets would expect to encounter at sites of injury. Indeed the effects were quite dramatic, showing greatest effects against the factor most responsible for activating clotting, the protein collagen. Using a range of approaches, they were able to demonstrate how LXR interacts with and inhibits the complex pathways of events that ultimately lead to platelet activation and thrombus formation.

Using a microfluidic experimental system, they were also able to study the effects of LXR ligand on the platelet thrombus formation process in flowing blood, where a dramatic 50% reduction in thrombus size was observed.

To understand the potential importance of this discovery for the prevention of thrombosis, the team turned to explore the effects of LXR ligand within the body, using a newly developed system to visualise thrombosis in the mouse. This sophisticated approach, allowed the visualisation and analysis of thrombosis as it occurs, and assessment of the ability of LXR ligands to reduced this. The ligands were found to reduce dramatically the size of the thrombosis by reducing the numbers of platelets within the clot and reducing the clot stability. Since inhibition of platelets could cause serious bleeding, this was also tested. Fortunately, only modest increases in bleeding were observed, pointing towards potential value in LXR as an anti-thrombotic target.

These exciting results indicate that drugs that are in development for other conditions, that target LXR may have another use in the prevention of thrombosis. This is the first step in the lengthy process between the understanding of platelet biology and the development of new drugs to prevent thrombosis. Progress may be boosted, however, due to existing drug development programmes.

This research was supported by grants from Heart Research UK, the British Heart Foundation, the Medical Research Council and the Wellcome Trust. Further details of the paper can be found in the journal article: Spyridon, M., Moraes, L.A., Jones, C.J., Sage, T., Bucci, G., and Gibbins, J.M. ‘LXR as a novel antithrombotic target.’ Blood 2011, 117, 5751–5761. This paper gained the attention of the media internationally, including over 50 international newspaper reports, and can also be found on the BBC news website at www.bbc.co.uk/news/health-12737549.
ICMR Highlights

Links between bacterial infection and thrombosis: mechanistic insights

Dr Simon Clarke is a lecturer in microbiology and his recent collaboration with Professor Jon Gibbins and Dr John Mckendrick has been rewarded with a British Heart Foundation (BHF) project grant to study the effects that Staphylococcus aureus (S. aureus) MRSA bacteria, has on blood clotting. In this article, Dr Clarke describes the key objectives of this study.

In addition to their roles in both normal clotting and circulatory disease, platelets can interact with bacteria that cause infections in humans. Research conducted by members of the ICMR is focused on understanding how, during bacterial infection, the normal functions of platelets are usurped. Not only does this provide us with crucial insight into the mechanisms and processes involved in development of important and sometimes life-threatening infectious diseases, but it may allow us to develop novel strategies to prevent or control inappropriate thrombosis and its associated conditions.

Bacterial interactions with platelets during infection can have a significant impact on disease progression and severity. Some bacteria can inhibit the normal activation of platelets that is required to stem blood loss and heal wounds. Some scientists contend that platelets are an important element of the body’s defences against infection, although this has not yet been confirmed. So prevention of platelet activation could potentially be a survival mechanism for pathogens. Paradoxically, certain bacteria can cause platelets to become activated at body sites and at times when normally they would not. This can cause the formation of small blood clots that can cause heart failure or strokes. Furthermore, such small clots can block blood capillaries, the tiny blood vessels that deliver blood to tissues in the body. This phenomenon, known as disseminated intravascular coagulation, is responsible for a serious life-threatening condition known as necrotising fasciitis, or more commonly ‘flesh eating disease’. The formation of tiny blood clots in capillaries causes them to become blocked, depriving tissues of blood and thus an oxygen supply causing the infected tissue to die (a process known as necrosis).

S. aureus, sometimes more commonly known as MRSA, is a bacterium that can cause life-threatening infections in humans and animals. Two features of this pathogen that we are most interested in are bacterial endocarditis and inhibition of wound healing. This pathogen is the most common cause of bacterial endocarditis, an infectious disease that can occur when S. aureus gains entry to the circulatory system. The resulting infection of the heart can cause it to fail, is difficult to treat with conventional antibiotic therapy, and consequently is often fatal. Similarly, this pathogenic bacterium can infect artificial heart valves and cause them to fail.

Key to the development of bacterial endocarditis is the ability of S. aureus to interact with platelets. The bacterium is able to induce platelets to form small aggregates that also contain the bacteria. These thrombi can attach to heart valves and prevent them from working properly. If they become detached, they can migrate through the circulation to the brain and cause a stroke. Using expertise in both the biology of S. aureus and platelets, our research is unpicking the mechanisms and processes involved in this type of platelet activation. We have uncovered roles for molecules on the surface of S. aureus which are essential, and others that merely contribute to the activation of platelets. Not only is it important to have a complete picture of how bacteria and platelets interact, in order to fully understand how disease initiates and develops, but also so that it can be treated or prevented.

The ability of S. aureus to inhibit wound healing is probably multi-faceted, involving inhibition of clot formation and tissue repair. Our research has discovered that a molecule produced by S. aureus, called lipoteichoic acid (LTA), inhibits platelet activation that is triggered by the normal physiological cues. We have shown that a receptor on the surface of platelets, known as PafR, recognises LTA and induces signalling within platelets that causes a generalised inhibition of activation. This phenomenon has not hitherto been described in platelets. We predict that LTA has a role to play in S. aureus mediated inhibition of wound healing, but are examining how its interaction with platelets inhibits their activation. The three-year project grant from the BHF will allow us to examine this further. The research will determine the effect that LTA has on platelets in finer detail. Understanding such events is crucial if we are to be able to exploit this avenue for controlling platelet activation.

This research is supported by a £195,000 Project Grant from the British Heart Foundation - Inhibition of platelet activation by Staphylococcus aureus lipoteichoic acid.
Fish oil fatty acids improve blood vessel elasticity

**Insights from the eFAIRE study**

The endothelium, a single layer of cells which line the blood vessel wall, plays a critical role in the maintenance of the vascular tone (elasticity of the blood vessels). This active tissue releases a number of substances, most notably nitric oxide, a potent vasodilator that causes smooth muscle relaxation and dilation of the blood vessels. Dysfunction of this process, characterised by a reduction in the elasticity of the blood vessels has been regarded as an early risk marker of cardiovascular disease. Evidence is now emerging that mechanisms involved in the control of vascular tone (also referred to as vascular reactivity) are influenced by dietary factors, with the type of fat eaten in the diet considered to be an important modulator.

Data published to date, provides reasonable evidence that the long chain n-3 polyunsaturated fatty acid fish oils, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have a beneficial impact on vascular reactivity when consumed as part of a high-fat meal (acute test meal studies) or following supplementation with fish oil capsules over 2–8 weeks (chronic studies). The mechanisms to explain the beneficial effects of fish oils on vascular function have been derived from longer-term supplementation studies and potential mechanisms include modulation of inflammation or levels of circulating lipids, production and stability of nitric oxide at the endothelium, as well as direct effects on the smooth muscle cell layer. It therefore appears that fish oils may have many interrelated properties which could act together to improve the health of the vasculature.

Conditions such as obesity and type 2 diabetes are increasing in prevalence in the UK population and are associated with an increased risk of developing cardiovascular disease. Studies in humans and animals have shown that artificially raising the level of circulating free fatty acids (also referred to as NEFA) to the levels usually observed in people with obesity and type 2 diabetes to cause vascular dysfunction. At present, little is known about how the type of fat that we eat in a meal can influence the fatty acid composition of circulating NEFA, and how this will impact on vascular function after a meal (termed postprandial vascular reactivity).

**Overview of the eFAIRE study**

In the paper published in the *American Journal of Clinical Nutrition*, researchers from the ICMR (Katie Newens, Abby Thompson, Kim Jackson and Christine Williams) determined the effects of drinks rich in either a) saturated fat or b) saturated fat with the addition of fish oil fatty acids (equivalent to 1.5 times a standard portion of oily fish) on the fatty acid composition of circulating NEFA and vascular reactivity. Fifty-nine healthy men and women attended the new vascular suite facilities in the Hugh Sinclair Unit of Human Nutrition on two different occasions to take part in this study. Using an experimental protocol, subjects consumed the fat containing drinks together with a heparin infusion to artificially elevate the levels of NEFA. Using ultrasound equipment purchased as part of the refurbishment of the clinical unit facilities supported by the Garfield Western Foundation, the elasticity of the large brachial artery in the arm was measured using flow-mediated dilatation. This gold-standard technique for measuring vascular reactivity involves the inflation of a blood pressure cuff on the lower arm to prevent the blood flow to the hand. After five minutes, the blood pressure cuff is rapidly deflated and the increase in blood flow to the hand initiates the production of nitric oxide which causes the arteries to dilate to accommodate the increase in blood flow (called reactive hyperemia). The diameter of the artery before, during, and after the inflation of the blood pressure cuff was visualised using ultrasound imaging to calculate the percentage flow mediated dilatation (an indicator of the elasticity of the blood vessels). Measurement of flow mediated dilatation was performed at baseline (in the fasting state) and four hours after the consumption of the fat containing drinks and heparin infusion.

**Significant observations**

The experimental protocol successfully raised NEFA levels to those observed in obese and diabetic individuals, and the fatty acid composition of the circulating NEFA measured at four hours reflected the fat-containing drinks consumed by the healthy men and women on the study days. Whereas the NEFA enriched in saturated fatty acids reduced vascular reactivity compared with the baseline fasting level, it could be completely reversed by a 3–5 fold increase in the enrichment of NEFA with fish oil fatty acids. A clinically significant 2.1% difference in flow mediated dilatation was observed between the study days using the saturated fat and saturated fat with fish oil drinks. In addition, a positive association between the baseline fatty acids present in NEFA measured at four hours (peak NEFA concentration) with changes in vascular reactivity, supports the notion that NEFA fatty acid composition is an important factor which determines how NEFA modulates vascular function.

**Conclusions**

Our findings have shown elevated NEFA levels to be associated with a reduction in vascular reactivity, thus supporting the view that raised NEFA levels that occur in obesity and type 2 diabetes could contribute to the increased incidence of endothelial dysfunction, and cardiovascular disease in these populations. It is unclear whether the improvement in flow-mediated dilatation (vascular reactivity) with fish oil fatty acids was associated with an increase in the bioavailability of nitric oxide at the endothelium or due to direct effects of fatty acid metabolites of EPA acid and DHA acid (such as fatty acid epoxides) on the smooth muscle cell layer. Further studies are needed to clarify these mechanisms and determine if they contribute to benefits observed in the longer-term supplementation studies.

This work was supported by the BBSRC (BB/G022181/1), Unilever Discover and the Foundation for Research Science and Technology (New Zealand). Further details of the paper can be found in the journal article: DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. Newens KJ, Thompson AK, Jackson KG, Wright J and Williams CM. *American Journal of Clinical Nutrition* 2011; 94:742–8.
This year, the ICMR Research Development Awards supported five undergraduate vacation studentships. A faculty-wide invitation was circulated to the undergraduate students giving them the opportunity to apply for a placement within the ICMR over the summer vacation. This feature gives a flavour of two of these studentships.

May Alexander’s project ‘Mathematical modelling of ERK2 phosphorylation and nuclear import in cardiomyocytes’ aimed to gain a deeper understanding of how enzyme activation occurs in cardiac cells.

Together with the project supervisors, Dr Marcus Tindall and Professor Angela Clerk, the specific aim of this project was to develop an initial mathematical model of extracellular signal-related kinase 2 (ERK2) signalling in cardiac myocytes (heart muscle cells) to test hypotheses regarding cytoplasmic and nuclear phosphorylation of ERK2 within the cell. The location and mechanism of phosphorylation and dephosphorylation of each enzyme involved in the signalling pathway were to be modelled and examined with the help of the experimental data, and the general behaviour of the system determined.

May: ‘On initially reading the description of the project, I was excited about the opportunity to experience research and work alongside leaders in the field. Heart cell research is an area that has interested me before, and learning how important the ERK pathway is, and how vital it is that we understand how it works, made me very eager to be able to contribute. The project description also mentioned the prospect of doing some work in the laboratory, and while I felt less confident there, it was really interesting to see and appreciate the amount and intricacy of the work needed to produce reliable experimental results.

‘I was excited about the opportunity to experience research’

Charlotte Bateson conducted a study ‘Investigating associations between body and organ fat with metabolic and cardiovascular disease risk’, supervised by Professor Julie Lovegrove.

Charlotte: ‘Having really enjoyed my disease-related modules in the first year of my degree (Pathology, Biochemistry and Human Physiology), I was very keen to gain some relevant experience during the summer. When I found out about the vacation Studentship I saw it as the perfect opportunity to gain some experience in an area that fascinates me, obtain an insight into what research entails in terms of a future career path, and be able to run a current project with the potential to achieve some very worthwhile results. After speaking with Professor Lovegrove, I was motivated by her enthusiasm for this project, and feel very fortunate that I was able to work on such a relevant topic linked to the rapidly increasing incidence of diabetes, obesity and cardiovascular disease.’

‘My project highlighted the importance of good time management, communication and organisational skills’

The pilot proof of principle study aimed to determine potential associations between body fat distribution and lipid deposits in the liver, and conventional and novel risk factors for cardiovascular disease and other metabolic disorders. I explored the use of the Magnetic Resonance Imaging (MRI)/Magnetic Spectroscopy (MRS) scanner for whole body fat distribution determination and intra-organ fat levels. With the help of Dr Shan Shen in the Centre for Integrative Neuroscience and Neurodynamics (CINN), these aims were achieved, broadening the scope of use of the MR scanner, which will be invaluable for application in future ICMR studies. We also now have a deeper understanding of the issues involved with scanning and testing participants over a broad range of body sizes (normal weight to morbidly obese).

‘During my research phase, I learnt a lot about the techniques including the MR technology (which required me to visit other researchers in the field to discuss their methodology) and flow-mediated dilation (the gold standard technique for the assessment of vascular function). Due to the involvement of several departments and enlisting the help of several colleagues, this project was not easy to coordinate. This, however proved an invaluable learning experience highlighting the importance of good time management, communication and organisational skills. I also gained skills in phlebotomy, sample analysis using an automated clinical chemistry analyser and interpretation of the clinical data.

I thoroughly enjoyed this project from start to finish, and look forward to participating in future studies of this nature.’
Building international links

Report from Drs Katrina and Andrew Bicknell on their ICMR-funded visit to Professor Mark Sussman’s laboratory, San Diego.

In recent years, the therapeutic potential of stem cells to repair the injured heart has been widely recognised and clinical trials have been rapidly undertaken to test the potential of various stem-cell-based approaches to improve cardiac repair. Results from the early pre-clinical and initial clinical trials, however, have largely been disappointing. Very few stem cells appear to home to the damaged region of the heart, differentiate into cardiac myocytes and integrate into the damaged myocardium, although improvement in cardiac function was observed. Moreover, the few cardiac myocytes that do integrate into the damaged region retain an immature muscle phenotype. Clearly, further research is required to understand the processes that promote the differentiation of stem cells into cardiac myocytes.

One of the strengths of the ICMR stems from the complementary research programmes driven by Drs Katrina Bicknell, Sam Boating, and Andrew Bicknell, and Professors Gavin Brooks, Angela Clerk, Ketan Patel and Peter Sugden. These research groups share a common focus and aim to understand the molecular and cellular events that modulate myocyte development, proliferation, differentiation and growth. To apply these research strengths and make an impact in the competitive area of cardiovascular stem cell research, a reliable source of cardiac progenitor cells must be available. The main purpose of this visit was to learn the techniques required to isolate, culture and characterise cardiac progenitor cells from adult mouse hearts from a leading expert in cardiac progenitor cell field, Professor Mark Sussman.

We spent two days visiting the laboratory of Professor Sussman at the San Diego State University Heart Institute, San Diego, USA. We observed the isolation of mouse cardiac progenitor cells, from enzymatic digestion of the heart to the separation of the rare cardiac progenitor cell population. We also discussed the culture and maintenance of isolated stem cell populations in detail. We had the opportunity to participate in the Sussman lab meeting before spending the rest of the visit meeting with Professor Sussman and his graduate students and postdoctoral researchers to discuss their ongoing projects, many of which focused on cardiac progenitor cells.

During the stay we also had the opportunity to visit Vala Sciences, a leading manufacturer of specialised microscope systems, mainly for the use of high throughput fluorescence-based assays. This company has developed an assay to quantify the phosphorylation and activation of both hormone sensitive lipase (HSL) and perilipins in adipose and cardiac tissue. We have previously collaborated with Dr Pat McDonough (VP of Biological Sciences at Vala), supplying him with purified pituitary peptides for use as agonists in some of their adipocyte assays. Following a tour of the research labs and microscope manufacturing facilities, discussions with key staff, and a presentation on our own work on HSL and perilipin, we were able to develop future collaborations with Pat and his research team at Vala Sciences.

Overall it was a very productive and useful visit. We are now fully equipped to start isolating mouse cardiac progenitor cells here at the ICMR. Once established at Reading, we also hope to modify the technique to allow the isolation of rat cardiac progenitor cells, which we propose would be less afflicted by the common problems observed in mouse progenitor cell cultures. We hope to be in a position to start work on our own cardiac progenitor cell lines in 2012.
Advances in biomedical research over the past three decades have led to a 50% reduction in the death rate of those suffering from cardiovascular disease.

Whilst this is a substantial achievement, the incidence rates for cardiovascular disease are increasing rapidly. It is recognised that conditions such as obesity, and obesity-related metabolic disorders such as type 2 diabetes, abnormal lipid metabolism, inflammatory disorders and imbalanced diet are at the centre of this increase.

Using a distinctive combination of research approaches and expertise the ICMR is successfully tackling this developing twenty-first-century healthcare crisis from a number of innovative directions, focusing on causes, prevention and treatment of these conditions. We aim to reduce the burden of cardiovascular and metabolic disease through our innovative research.

**Highlights**

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