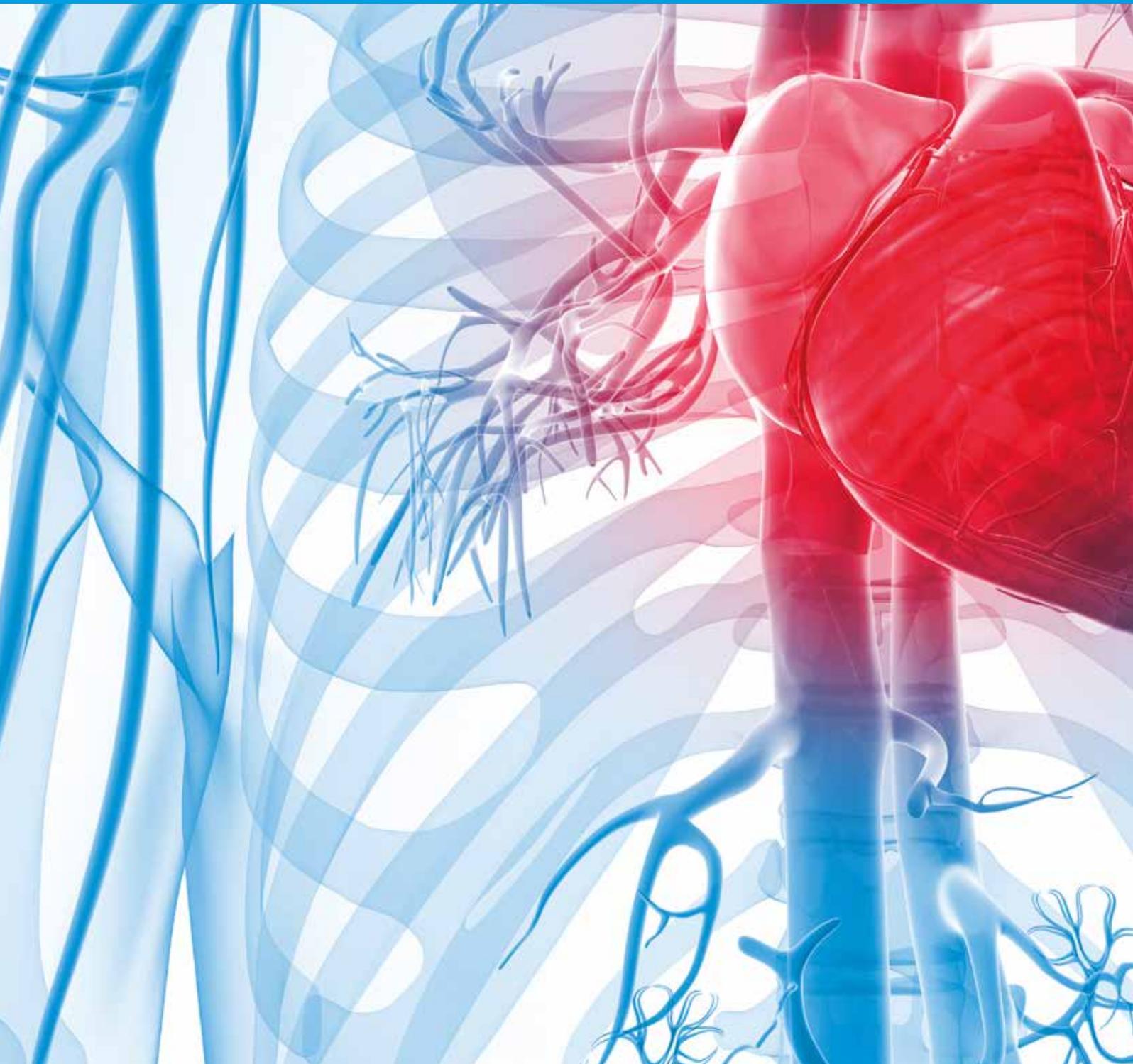


# HIGHLIGHTS





Professor Jon Gibbins



Professor Julie Lovegrove

The ICMR is a multidisciplinary centre that brings together scientists from a wide range of research fields to work to better understand the causes of cardiovascular diseases and the underlying obesity-related metabolic diseases from which they develop. The activities are broad and wide reaching and this publication aims to give a taster of these activities and examples of our future research directions.

## What's inside?

- 3 News
- 4 New IMCR imaging facility
- 6 Research highlights
- 10 In conversation
- 11 ICMR Travel Award winners

# WELCOME

Welcome to the second edition of *Highlights*. Within this edition we aim to bring you up to date with recent advances made through ICMR research to better understand cardiovascular and metabolic disease, its development, prevention and treatment.

Since the first issues of *Highlights* there have been a number of exciting developments that have enabled the further expansion of the ICMR. This includes a number of new academic appointments increasing our capacity to explore cardiovascular cell biology and physiology, diet genes and cardiometabolic health, and haemostasis and thrombosis. One of our new researcher leaders, Dr Lucia Stefanini is 'In conversation' on page 10.

There have been many recent research breakthroughs across the spectrum of our research, and some specific examples, from genetic epidemiology, atherosclerosis, dietary fats to systems biology and innovative use of mathematics, are presented on pages 6–9.

A particular highlight for us has been the establishment of the ICMR Cardiovascular Imaging Facilities. In order to remain at the 'cutting-edge' of research we have expanded dramatically our imaging facilities across the spectrum of our research incorporating cell and molecular, animal and human imaging (see pages 4 and 5). This significant development has been made

possible by substantial investment by the British Heart Foundation, the Wolfson Foundation, the Medical Research Council, the Garfield Western Foundation, some generous private donations and the University of Reading.

The Imaging Centre is an important investment in our scientific future. This marks the beginning of a very exciting phase in the development of the ICMR with a substantial number of new academic groups joining us in the next year and the initiation of a process to develop new infrastructure: laboratory facilities and human study and clinical facilities, allowing the closer integration of researchers to provide new and innovative insight to the challenge of reducing the burden of cardiovascular disease.

We hope that you enjoy this brief excursion into our work. If you are interested in knowing more or even contributing as a volunteer to one of our studies, please do refer to our website and contact us directly.

# NEWS

## BREAKTHROUGH: THROMBOSIS – ‘MIND THE GAP’

Researchers within the ICMR have discovered that platelets possess specialised pore forming proteins, connexins, which form structures known as hemichannels and gap junctions that allow molecular communication between these cells. Dr Sakthivel Vaiyapuri and colleagues have revealed import functions of these proteins in the control of blood clotting and thrombosis. The results of this study were published in *Circulation* and *Nature Communications*. Work is ongoing to establish how the functions of these proteins in platelets are controlled since connexins may represent new anti-thrombotic drug targets.

## BREAKTHROUGH: STATINS – THE CLOT THICKENS

Statins are usually used to reduce the amount of cholesterol in our blood circulation, but they are also recognised to have cholesterol-independent beneficial effects. In a recent study published in the journal *Blood*, Dr Leo Moraes and colleagues discovered that some statins reduce platelet reactivity through a mechanism linked to the stimulation of the inhibitory platelet receptor PECAM-1. In their work, the effects of statins on PECAM-1 function were shown to inhibit thrombosis.

## CHALLENGING DIETARY RECOMMENDATIONS FOR CARDIOVASCULAR PREVENTION

Dairy foods contribute to a significant proportion of dietary saturated fat in the UK diet, yet high milk consumers have a lower cardiovascular disease mortality and type 2 diabetes prevalence. A high profile research study funded by the MRC aims to determine the impact of modified dairy products produced from low saturated fat/ high monounsaturated fat milk on novel and traditional risk factors for cardiovascular disease. See page 9 for details.

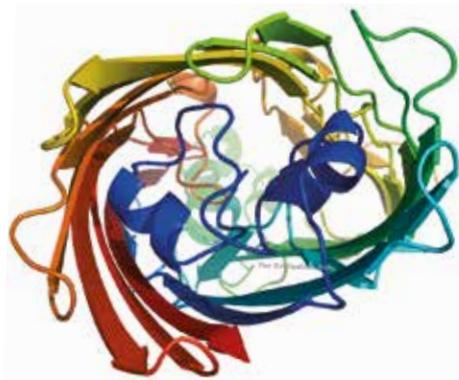
## IS FIVE-A-DAY ENOUGH?

While consumption of fruits and vegetables (F&Vs) are associated with a lower cardiovascular disease risk, the optimal type and daily intake is unknown, resulting in inconsistent global public health recommendations for F&V intakes. Results from the FLAVANoids University of Reading Study (FLAVURS) suggest that five portions of F&V may not be optimum to lower CVD risk, and that flavonoid-rich F&Vs in particular may be important in maintaining vascular health (see page 9 for details).



## PAVING THE WAY FOR STRUCTURE-DRIVEN VACCINES

Dr Kim Watson and her group recently solved the structure of MOMP, a major outer membrane protein from *Chlamdophila pneumoniae*, a potential vaccine target for infections leading to cardiovascular events and respiratory diseases.



MOMP shows an occluded barrel, which has implications for function

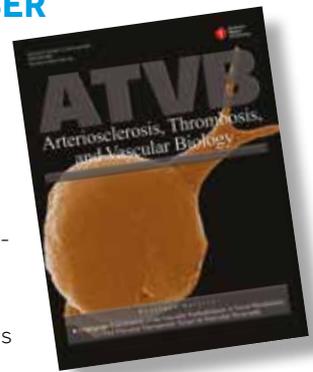
## HERPETOLOGY MEETS HAEMATOLOGY...

Viper venom proteins exert their envenomation effects by mainly affecting the cardiovascular system. Researchers at the ICMR have had notable success in exploring the functions and effects of specific venom proteins. By understanding the complex mixtures of active components within

venoms the aim is develop safer and more effective measures to treat the life-threatening symptoms of envenomation. This work and an associated survey of snakebite incidence, mortality and morbidity, has gained media attention as far afield as the *New York Times* and the *Times of India*.

## AWARD FOR ICMR MEMBER

A scanning electron micrograph of a platelet at an initial stage of activation was published as a cover image in the journal *Arteriosclerosis, Thrombosis, and Vascular Biology* in December 2013. This image was selected as the *Outstanding Cover 2013* in the ATVB conference in Toronto (May 2014).



## NEW EDITORIAL ROLES FOR ICMR ACADEMICS

Six new appointments broaden the ICMR's role in the publication of high-quality science in a wide range of cardiovascular and metabolic research disciplines:

Prof Parveen Yaqoob joined the editorial board of the journal *Atherosclerosis*, Dr Keith Foster joined the Editorial advisory panel of the *Journal Clinical Science*, Dr Gunter Kuhnle is now part of the editorial board of *Nutrition and Aging*, Dr Jonathan Swan of the editorial board of *Microbiome*, Dr Kim Jackson of the Editorial board British Journal of *Nutrition*, and Prof Jon Gibbins joined the editorial Board of the *British Journal of Pharmacology*.





# NEW ICMR IMAGING FACILITY

The last few months has been marked by the launch of our new Imaging Facilities. These state-of-the-art systems will allow us to peer into cells at unparalleled levels of resolution, to look inside human donors and to explore the mechanisms of cardiovascular and metabolic disease in animal models of human disease.

## IMAGING CELLS AND MOLECULES

The new ICMR confocal microscopy suite brings cutting edge cellular imaging capabilities to Reading. Housed in the Lyle tower, the suite consists of two fully motorised **Nikon A1-R confocal microscopes**, each capable of four colour imaging and both equipped with environmental chambers allowing live cell imaging. These microscopes support an extremely wide range of dyes and fluorophores and are adaptable, so no matter what requirements you have the microscopes should be able to handle it! In addition to this flexibility, each microscope also brings its own unique capabilities. One of the systems has been adapted for high speed image acquisition using the fastest scanners and most sensitive detectors available. This will allow users to track very rapid events in cells or to capture images with very low laser power. The other system provides super-resolution imaging capabilities. Using a technique called **N-STORM** images can be captured at much higher resolutions than is possible with standard confocal microscopes with a resolution of 20–30 nm achievable. This is one of only three such systems in the UK and will allow many new novel approaches to investigating the behaviour of cells.

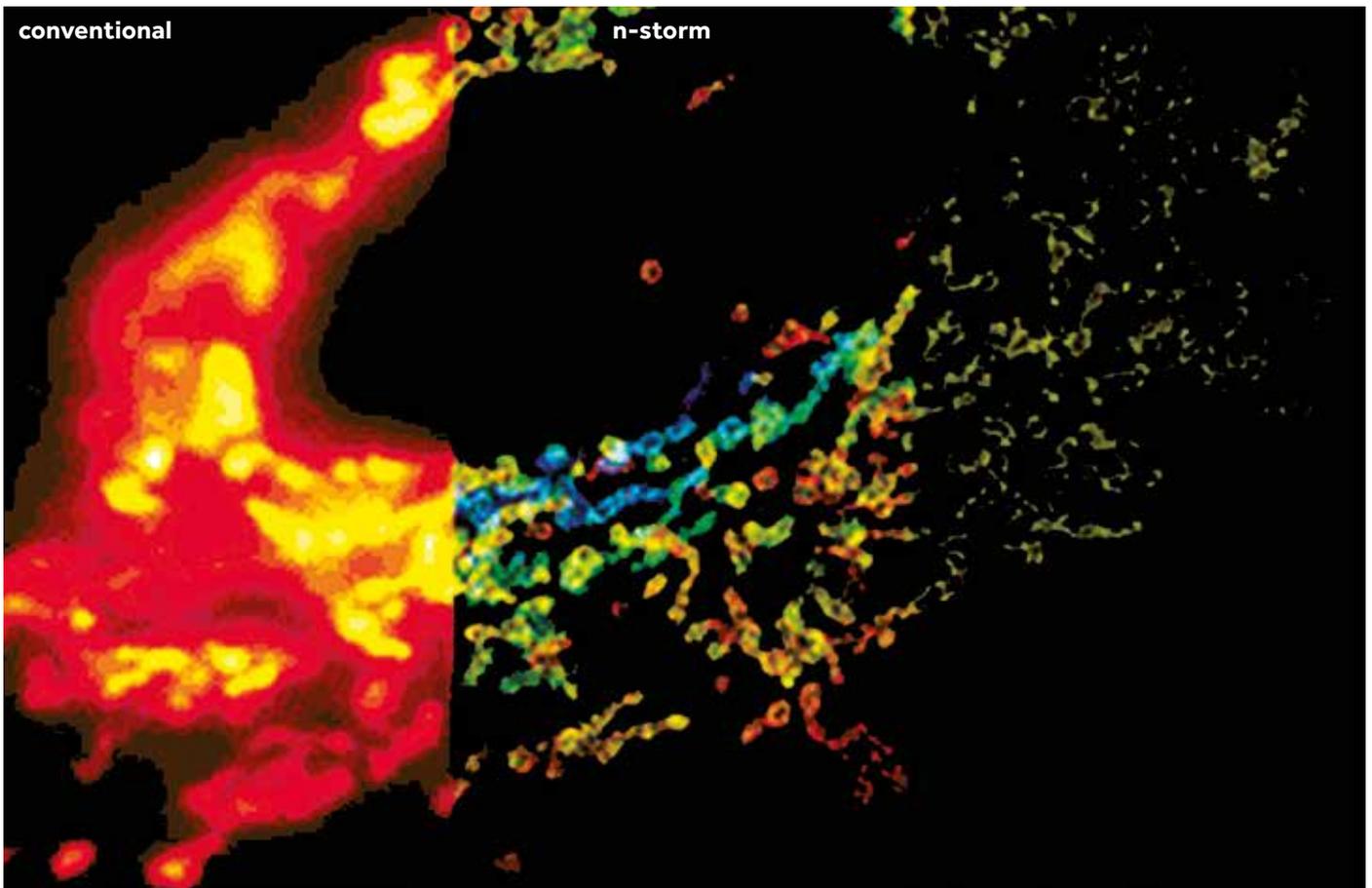
## IMAGING DISEASE DEVELOPMENT

Research has shown that body composition is a key component of health and future disease risk. As a result, there is considerable interest in methods to accurately monitor and assess changes in body composition. **Dual x-ray absorptiometry (DXA)** is a non-invasive gold standard technique for the measurement of bone mineral density, an indicator of bone health. The ICMR imaging facilities now include a **Lunar iDXA** which offers research grade image resolution to deliver crisp, high resolution images of the vertebrae.

Historically DXA has only been used to assess bone mineral density. However the Lunar iDXA can also accurately measure total body composition, assessing bone, fat and lean tissue mass within one short body scan. Automated software calculates regional percentage body fat and BMD, and the percentage of body fat distributed around the stomach and hips. The software package, CoreScan, also determines the mass and volume of visceral fat within the abdomen, an indicator of conditions such as the metabolic syndrome and type II diabetes.

Mirror imaging software allows scanning of study participants up to 204 kg (32 stones) and 1.93 m (6ft 4 inches) in height. The DXA scanner uses a low dose of ionising (X-ray) radiation to determine bone, fat and lean tissue mass so this technique is not suitable for use in studies with pregnant women.

Blood vessel (vascular) dysfunction is becoming increasingly recognised as an early marker of cardiovascular disease risk and a critical modifiable event in the development of atherosclerosis. Numerous studies have now highlighted the prognostic value of 'in vivo' measures of vascular reactivity of both the coronary and peripheral arteries, in predicting future coronary events. The ICMR imaging facilities now includes two Philips CX50 portable ultrasound machines utilising SonoCT imaging technology, each equipped with L12-3 and L15-7io linear array transducers to provide crisp, high resolution images of the human vasculature. Current applications include the imaging of the diameter of the brachial artery using the L15-7io transducer to assess nitric oxide mediated vasodilation during the flow mediated dilatation technique and measurement of the carotid intima media thickness.



## IMAGING DISEASE PROCESSES

### Intravital microscopy

Over the last four years, with support from the MRC, we have established a state-of-the-art intravital microscopy system, which allows the **visualisation of fluorescence signals *in vivo***. This comprises an upright epi-fluorescence microscope with a rapid light source wavelength changer, automated shutter control, automated stage, an image intensifier and a high-speed digital camera. This allows simultaneous real-time analysis of up to four different fluorophores, and SlideBook software is available to coordinate the components of the system, acquire data and for offline data analysis.

The system has been established within the Haemostasis and Thrombosis Group to study thrombosis, which may be triggered using an ablation laser that is directed through the microscope objective lens. The success of this system to measure thrombosis, has led, as part of our recent imaging facility project to the addition of a high speed spinning disk confocal scanner together with multiple laser lines and a high sensitivity camera. This will enable time resolved analysis of thrombus formation in three dimensions allowing the structure and stability of thrombi and components and processes within to be analysed at levels of

detail that were until recently unimaginable. While initially designed with thrombosis studies in mind, this unique system combining intravital microscopy with high-speed confocal analysis will impact on a wide range of ICMR research.

### High frequency ultrasound VEVO 2100

The **VEVO 2100** is the state of the art system for in vivo imaging studies in rodents and other small species. The system is currently equipped to perform studies of cardiac and vascular function. In addition to the cardiovascular analysis software package, the VEVO 2100 includes a 3D mode, which allows volumetric analysis of the whole heart or tumours, for quantification and assessment, and a general echography package that allows evaluation of abdominal organs as well as tumours, very useful for example in the field of cancer research.

The system has been customised to expand its capabilities through software and hardware upgrade and to evaluate micro vessel dynamics (e.g. blood flow) with the use of contrast agents, which is important in disease processes such as cardiac and vascular disorders.

**Above** The Nikon A1-R confocal microscope with N-STORM capabilities (right) provides dramatically enhanced resolution compared to conventional optical microscopes (left).

**Top left** Lunar iDXA. Whole body scanner is used to assess bone, fat and lean tissue mass with low doses of radiation.

**Below** VisualSonics Vevo 2100 Imaging System is employed for in vivo visualization, assessment, and measurement of the cardiovascular system for small animal phenotyping.



# RESEARCH HIGHLIGHTS

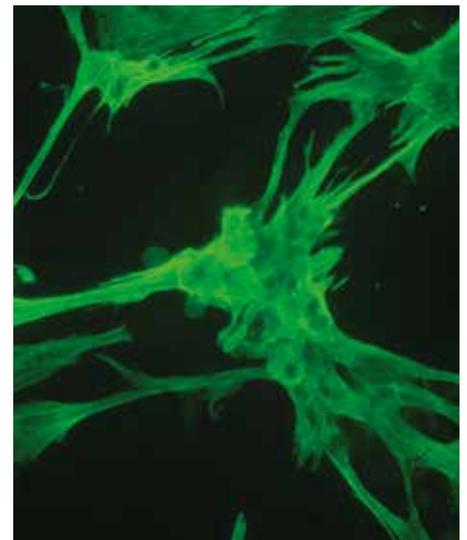
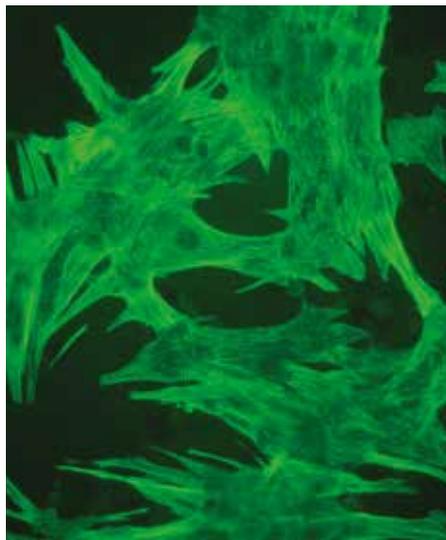
## MATHEMATICS, microRNAs AND HEART FAILURE

Marcus Tindall and Angela Clerk

A combination of mathematical and cell biological techniques elucidated how miRNAs regulate the expression of ATF3 a major hub in cardiac myocyte response to stress and a potential target for cardiac hypertrophy and heart failure.

The function of cells within our bodies is controlled by proteins. Molecules known as messenger RNAs (mRNAs) carry the information for protein synthesis from the genes to the ribosomes and allow protein expression. In recent years a new type of RNAs, known as microRNAs (miRNAs), have been discovered and they are thought to be involved in the negative regulation of protein expression.

Work in Prof Angela Clerk's group at the ICMR has recently shown that in cardiac myocytes (heart cells) there is a clear difference in the time varying levels of mRNA and protein for a transcriber known as *Activating Transcription Factor 3* (ATF3)<sup>1</sup>. ATF3 is a master regulator of cell response to stress conditions, such as injury, ischemia, ischemia/reperfusion or chemical toxin, and is known to be important in many disease states including inflammation, cancer and cardiovascular disease. Recent studies identified ATF3 as a nexus in cardiomyocyte hypertrophy as it is required to facilitate the full and proper growth of heart cells. Its protective effect in pathological hypertrophy in the heart suggests that ATF3 may be an effective therapeutic target for cardiac hypertrophy and heart failure<sup>2</sup>, thus it is important to uncover the mechanisms that regulate ATF3 expression at the mRNA and the protein level. For this purpose Prof Clerk and Dr Marcus Tindall employed a math-



ematical modelling approach to examine a number of hypothetical scenarios as to how other cellular proteins and transcribers may or may not interact with ATF3 DNA to produce the experimentally observed levels of ATF3 mRNA and protein. Dr Tindall developed a series of mathematical models to explore which of these hypotheses may produce results similar to those observed experimentally. Through joint work recently published in the journal *PLoS Computational Biology* in May 2014<sup>3</sup>, Prof Clerk and Dr Tindall were able to disprove a range of biologically plausible scenarios, some previously considered in the literature, others not. For instance, the reported hypothesis that the ATF3 protein may be controlling the transcription of its own gene by a negative feedback mechanism was refuted. Among the possible scenarios they postulated the likelihood that miRNAs may be able to regulate ATF3 mRNA levels by binding and reducing the amount of free mRNA molecules within the cell. Results from the formulated mathematical model were in strong agreement with the experimental data, even under considerable variation in the levels of other proteins in the cell, and strongly suggest that miRNA may work in this way to control ATF3 mRNA levels not

Cardiac myocyte hypertrophy induced by endothelin-1 is disrupted in the absence of ATF3. Rat neonatal ventricular myocytes were infected with adenoviruses (left) or adenoviruses encoding antisense ATF3 (right), then exposed to endothelin-1 (24 h). Cells were fixed and stained with antibodies to troponin T.

only in heart cells but also in other cellular systems. Prof Clerk and Dr Tindall are currently considering ways to extend their work to such systems and further understand the role of miRNA regulation.

This research was supported by the British Heart Foundation.

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- 2 Zhou H et al. Activating transcription factor 3 deficiency promotes cardiac hypertrophy, dysfunction, and fibrosis induced by pressure overload. *PLoS One*. 2011;6(10):e26744.
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# ATHEROSCLEROSIS AND THE OXIDATION OF LOW DENSITY LIPOPROTEIN IN LYSOSOMES: THE ENEMY WITHIN

David S. Leake

Atherosclerosis is the underlying cause of coronary heart disease and thrombotic strokes and is therefore the leading cause of death in the world.

Most cholesterol in plasma is carried in low density lipoprotein (LDL). The oxidation of LDL (a process caused by free radicals, analogous to milk becoming rancid) is widely believed to be important in atherosclerosis. The oxidised LDL hypothesis proposes that cells in the arterial wall oxidise LDL in the extracellular space (interstitial fluid) and then macrophages take it up rapidly, leading to the accumulation of cholesterol in macrophages and the formation of atherosclerotic lesions. A problem with this hypothesis is that the oxidation of LDL is inhibited by low concentrations of serum or interstitial fluid and that large clinical trials have shown no protection by antioxidants against cardiovascular disease.

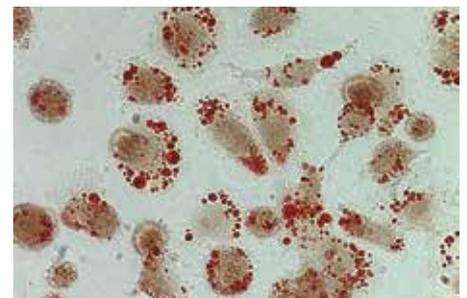
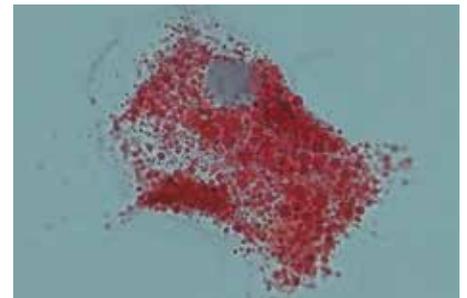
We have shown for the first time that macrophages oxidise LDL in their lysosomes, rather than, or in addition to, the extracellular fluid. This raises the possibility that lysosomes are the major site of LDL oxidation in atherosclerotic lesions. Atherosclerotic lesions are inflammatory sites and contain enzymes, such as sphingomyelinase, that are increased in inflammation. We proposed the hypothesis that LDL is acted on by these enzymes in the interstitial fluid of atherosclerotic lesions and becomes unstable and aggregates. The aggregated LDL is rapidly endocytosed by macrophages and then oxidised within lysosomes due to the acidic pH (pH 4.5) of these organelles and the presence of redox-active iron inside them. The lysosomes are the body's Achilles' heel as regards redox-active iron, as they are one

of the very few sites where iron is allowed to become redox active. The evidence to support this hypothesis was obtained using both morphological and biochemical techniques and applied to both mouse macrophages and human blood monocyte-derived macrophages. The iron chelator desferrioxamine, which is pinocytosed by cells and delivered to lysosomes, inhibits LDL oxidation, indicating the importance of iron in the lysosomal oxidation of LDL. The oxidation is also inhibited by chloroquine, a drug that increases the pH of lysosomes, indicating the importance of an acidic pH. The level of oxidation in lysosomes is extensive, as shown by the production of ceroid, an advanced oxidation product consisting of oxidised and polymerised lipid-protein complexes. It has been known for many years by pathologists that ceroid is found in cholesterol-laden macrophages in human atherosclerotic lesions. We also showed using spectrophotometry that iron can oxidise LDL effectively at the lysosomal pH of 4.5, but not at pH 7.4.

**OUR FINDING PROVIDES A RATIONALE FOR USING ANTIOXIDANTS TARGETTED TO LYSOSOMES AS A NOVEL THERAPY FOR ATHEROSCLEROSIS AND WE ARE CURRENTLY WORKING ON THIS.**

These findings provide a novel mechanism to explain how oxidised LDL is formed in atherosclerotic lesions in a sequestered intracellular environment in the presence of seemingly overwhelming antioxidant protection in the extracellular space. It may help to explain why the large clinical trials of antioxidants showed no benefit in cardiovascular disease, if the antioxidants could not enter lysosomes effectively or were consumed in the lysosomes and not replenished. In fact, we have found that vitamin E, the main antioxidant used in

the clinical trials, actually increases, rather than decreases, the initial oxidation of LDL by iron at acidic pH. Our finding provides a rationale for using antioxidants targeted to lysosomes as a novel therapy for atherosclerosis and we are currently working on this.



Low density lipoprotein (LDL) was incubated with sphingomyelinase to aggregate it and then incubated with macrophages derived from human blood (top picture) or human macrophage-like THP-1 cells (bottom picture). The cells were stained with a red dye (Oil Red O) to show that they had accumulated lipid (mainly esterified cholesterol), which was contained within lysosomes in the cells.

This research was supported by the British Heart Foundation.

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# GENETIC RESEARCH CLARIFIES LINK BETWEEN HYPERTENSION AND VITAMIN D DEFICIENCY

## Findings from the D-CarDia Collaboration

**Vimal Karani**

Dr Vimal Karani is a Lecturer in Nutrigenetics at the Department of Food and Nutritional Sciences, University of Reading and an Honorary Lecturer in Genetic Epidemiology at the University College London.

The D-CarDia (Vitamin D, Cardiovascular and Diabetes) collaboration investigates the causal relationship between vitamin D levels and the risk of cardiovascular disease-related traits such as obesity, blood pressure, hypertension, diabetes and inflammation. The causal relationship has been established using a genetic approach called 'Mendelian Randomization' where the causality is inferred from associations between genetic variants that mimic the influence of a modifiable environmental exposure (e.g. vitamin D) and the outcome of interest (e.g. cardiovascular disease-related outcomes such as hypertension). Because gene variants do not change over time and are inherited randomly, they are not prone to confounding and are free from reverse causation. If vitamin D concentrations are causally involved in determining blood pressure or the risk of hypertension, then the genetic variants that affect circulating concentrations of vitamin D could be expected to affect blood pressure and

hypertension risk. This assumption was valid for at least two of the genes that affect vitamin D, namely *CYP2R1* (encoding cytochrome P450, family 2, subfamily R, polypeptide 1) and *DHCR7* (encoding 7-dehydrocholesterol reductase). These genes function upstream of vitamin D production and affect vitamin D synthesis or substrate availability. Hence, the genetic markers from these two genes were used as proxy markers for lifelong differences in vitamin D status to test for a causal association with blood pressure and hypertension.

The first publication from the D-CarDia collaboration on 42,024 individuals had shown that obesity leads to lower vitamin D status while any effects of low vitamin D status on obesity are likely to be small<sup>1</sup>. To date, the D-CarDia collaboration comprises nearly 108,000 individuals from the UK, central and southern Europe, North America, Scandinavia and Finland. These data were used for examining the causality between low vitamin D status, blood pressure and hypertension. To further increase the statistical power of the study, the results were meta-analysed (combining the results from different studies in order to identify patterns, sources of disagreement or other interesting relationships among those results) with the summary data from the International Consortium for Blood Pressure (ICBP), Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) and Global Blood Pressure Genetics (Global BPGen) consortia (total of 150,000 subjects).

In meta-analyses of data from these consortia, every 10% relative increment in genetically instrumented vitamin D concentration was associated with 0.29 mmHg lower diastolic blood pressure (P=0.01), a 0.37 mmHg lower systolic blood pressure (P=0.05) and an 8.1%

reduced odds of hypertension (P=0.002). The estimated effect was relatively strong for hypertension, with the D-CarDia data suggesting 8% reduction in the risk of hypertension for each 10% increment in vitamin D concentrations. The results from this Mendelian randomisation study<sup>2</sup> provide evidence that increased vitamin D concentrations are causally associated with

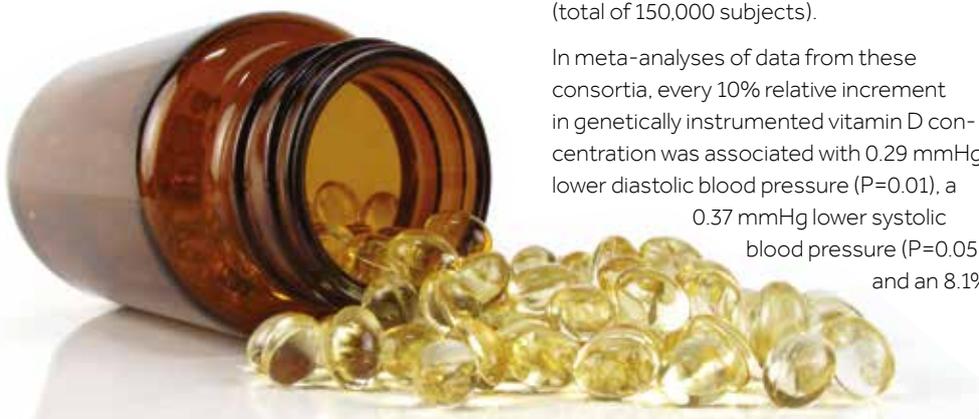
**THE RESULTS FROM THIS MENDELIAN RANDOMISATION STUDY<sup>2</sup> PROVIDE EVIDENCE THAT INCREASED VITAMIN D CONCENTRATIONS ARE CAUSALLY ASSOCIATED WITH REDUCED BLOOD PRESSURE AND HYPERTENSION RISK.**

reduced blood pressure and hypertension risk. Furthermore, these findings strengthen the case for appropriately powered, well-designed randomised clinical trials to investigate the necessary vitamin D doses and appropriate target groups for the prevention or treatment of hypertension.

Low vitamin D status is common throughout the western world; hence, the public health implications of these findings are notable. In view of the costs and side-effects associated with antihypertensive drugs, the potential to reduce hypertension by vitamin D is very attractive.

### References

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# DO FLAVONOID-RICH FRUITS AND VEGETABLES IMPROVE VASCULAR FUNCTION?

Anna Macready

Fruits and vegetables (F&Vs) have long been known to support healthy cardiovascular (CV) and metabolic function, however public health messages vary between countries with little consensus on type or amount of F&Vs necessary for optimal health.

F&Vs contain vitamins, minerals, nitrates and flavonoids. Flavonoids, found in high quantities in certain F&Vs such as berries, citrus fruits, peppers, onions and broccoli, may improve key markers of CV risk, including vascular reactivity, inflammation and oxidative stress. However, little is known about the amount and types of F&Vs required for CV health when delivered as whole foods, and there is a need to identify optimal intakes for public health guidance. The FLAVonoids Vascular University of Reading Study (FLAVURS), funded by the Food Standards Agency was designed to address these questions.

The FLAVURS project, led by Professor Julie Lovegrove was carried out in the University of Reading's Hugh Sinclair Unit of Human Nutrition. We recruited 174 low consumers of F&Vs, aged 26–70, who were at risk from CV disease, from the local Reading area. During this 18 week study, we asked our participants to consume either flavonoid-rich or flavonoid-poor F&Vs in increasing doses: two, then four, then six portions per day in addition to their usual diet, over a period of 18 weeks. We also asked a control group to maintain their normal diet and lifestyle and to include no additional F&Vs.

We reported improvements in vascular reactivity and inflammatory markers, key indicators of CV risk, for men consuming

a total of six portions of high flavonoid F&Vs per day. We also found improvements in plasma nitric oxide (NO) for both men and women consuming a total of seven portions of high flavonoid F&Vs. NO enhances vascular reactivity and may help to slow the progression of atherogenesis, a key contributor to CV disease. Moreover, participants on the F&Vs diets maintained healthier vascular function and NO levels relative to controls during the course of the study.

Results suggest that F&Vs in general provide benefits for healthy vascular function. High flavonoid F&Vs were shown to be particularly beneficial, with men in particular benefiting from eating at least six portions a day.

Macready AL et al. FLAVURS Study Group. Flavonoid-rich fruit and vegetables improve microvascular reactivity and inflammatory status in men at risk of cardiovascular disease – FLAVURS: a randomized controlled trial. *Am J Clin Nutr.* 2014 Mar;99(3):479–89.

## REDUCING CARDIOVASCULAR DISEASE RISK THROUGH REPLACEMENT OF SATURATED FAT IN MILK AND DAIRY PRODUCTS: THE RESET STUDY

Oonagh Markey

There is an urgent need to focus on modifiable risk factors for CVD, including a high intake of dietary saturated fatty acids (SFA). While reduction of dairy products is often recommended for lowering SFA intake, this strategy has been strongly challenged because cow's milk is a vital source of essential micronutrients and proteins, some of which have been associated with beneficial hypotensive effects. Furthermore, prospective evidence suggests that high milk consumption is linked to lower risk of CVD. By contrast, altering the fatty acid profile of milk is a potential sustainable means of reducing SFA intake at a population level by removing SFA from the food chain, whilst retaining the beneficial aspects of milk.

A BBSRC DRINC funded study led by Professor Givens demonstrated that including processed rapeseeds in the dairy cow diet can reduce SFA content in milk from 70 to 45–60 g/100g total fatty acids (TFA) while increasing *cis*-mono-unsaturated fatty acids (*cis*-MUFA) from 20 to 33 g/100g TFA. However, there is a dearth of research examining the impact

of modified SFA-reduced dairy products on holistic risk markers for CVD, including vascular function, pro-inflammatory mediators and postprandial lipaemia.

In an attempt to reduce this knowledge gap, the MRC-funded RESET (REplacement of SaturatEd fat in dairy on Total cholesterol) Study, led by Prof Lovegrove, is investigating the impact of consumption of modified dairy products, that have a substantial proportion of SFA replaced with *cis*-MUFA, on risk factors for CVD. We will determine whether modified milk, cheese and butter intake will improve traditional and novel CVD risk factors, relative to commercially available products. The three-year project, which started in late 2013, will inform public health policy on optimum dietary strategies to prevent or delay the onset of CVD.

**We are currently recruiting participants for the RESET study. We are looking for non-smoking men and women aged between 25 and 65 years to take part. If you are interested in finding out more please email: Dr Oonagh Markey: [o.markey@reading.ac.uk](mailto:o.markey@reading.ac.uk)**



Dr Lucia Stefanini, formerly Research Assistant Professor in Biochemistry and Biophysics at the University of North Carolina (Chapel Hill), was recently appointed Senior Research Fellow in the School of Biological Sciences as part of the University's Academic Investment Project. In this recent interview she tells us about her background, research and plans for the future.

### **Congratulations on your new appointment! What are your research interests?**

I am interested in the molecular mechanisms by which platelets contribute to health and disease. Platelets are the blood cells that survey the integrity of blood vessels. When we are injured platelets become active and bind to each other to plug the hole and prevent the loss of blood. For our health it is very important that platelet activation is tightly controlled. When platelets numbers are too low or do not activate properly this may cause bleeding, but when platelets are too active they may form aggregates that occlude a vessel and stop the supply of blood and oxygen to important organs such as the heart or the brain. As of today, heart attack and stroke are major causes of morbidity and mortality in our society and we haven't yet found a way to prevent thrombosis without causing unwanted bleeding.

### **What will you be investigating here at the University of Reading?**

My research focuses on a class of small G proteins called RAPs that are the 'molecular

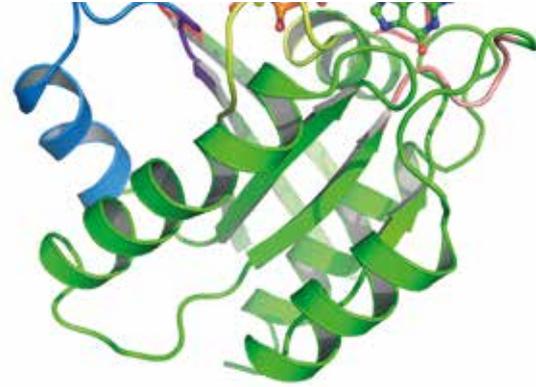
switches' of platelet activation. During my postdoctoral training I studied how these proteins are 'switched on' at a site of injury, now I am investigating how RAPs, once active, 'switch on' the platelet itself. I think that if we understand the 'switch' we can learn how to control it and potentially fine-tune it to find the perfect balance between bleeding and thrombosis.

### **Where did you study and train?**

I began my studies at the University of Pavia, Italy, where I completed my PhD in Biochemistry in the laboratory of Prof. Mauro Torti, an expert on platelet signalling. In 2008 I moved to the United States for my postdoctoral training and I joined the laboratory of Prof. Wolfgang Bergmeier first at Thomas Jefferson University (Philadelphia) and later at the University of North Carolina (Chapel Hill). My experiences in the two institutions had a complementary role in my education. In Philadelphia I had access to various models of cardiovascular disease and I had the opportunity to interact with clinicians and world-experts in the platelet field, which gave me more insight in the role of platelets in health and disease. At UNC I worked mainly with biochemists and biophysicists with a strong focus on small G proteins like RAP so I acquired new molecular tools to study these very interesting proteins.

### **How does your work fit in with other research in the ICMR and the research priorities of the University as a whole?**

Cardiovascular and metabolic diseases are the greatest problems of our society and the University has recently decided to invest in this field and I was head-hunted for this specific reason. The ICMR unites research groups that employ very diverse set of techniques and study different cell models but with a common goal to understand and solve cardiovascular and metabolic diseases, thus the potential for collaborations are astounding.



# In conversation

with **LUCIA STEFANINI**

### **What are the main challenges you face regarding your research?**

The greatest difficulty in studying platelets is the fact that you can't manipulate their genetic makeup and we have to rely on mouse models to study the effect of a protein mutation or deficiency. According to the latest proteomic studies there are almost 5000 proteins in platelets and we can't generate a mouse model for each. I think the challenge of our generation will be to find new strategies to study protein functions and dissect signalling pathways in a more efficient and less costly manner.

### **What have been your proudest achievements?**

My proudest achievement is the characterization of a protein called RASA3 in platelets. For 30 years we have used inhibitors of the platelet ADP receptor P2Y<sub>12</sub> to prevent the formation of occlusive thrombi and about 10 years ago it was demonstrated that P2Y<sub>12</sub> controls RAP, the 'molecular switch' I was talking about, but nobody knew how. My recent work demonstrates that RASA3 is the missing link between P2Y<sub>12</sub> and RAP and elucidates for the first time how P2Y<sub>12</sub> inhibition affects thrombus size and stability.

### **Looking ahead what are your aims for the next few years?**

I am in the process of applying for a British Heart Foundation Intermediate Basic Science Fellowship and I am looking forward to establishing my own research group at Reading. In addition I have many ideas for collaborations with ICMR colleagues. I am really excited about a new project in collaboration with Dr Chris Jones where we will employ the super-resolution microscope that has recently been incorporated in the ICMR imaging facility to study the mechanisms regulating integrin clustering.

**Top** Archetypical structure of small G proteins like RAPs, the molecular switches of platelets

## ICMR TRAVEL AWARD WINNERS

An integral part of ICMR mission is to train young investigators and to promote their attendance to international and national conferences. For this purpose last year the ICMR gave travel awards to nine candidates from various fields of cardiovascular and metabolic research. The following are the list of ICMR travel award winners:



**Chris Jones** is a Senior Research Fellow who studies genetics and genomics to gain insight into platelet signalling. Chris used this travel award to attend the XXIV Congress of the International Society on Thrombosis and Haematosi in Amsterdam, Netherlands.



**Michael Schenk**, a PhD student supervised by Prof Jon Gibbins, was awarded an ICMR grant to attend and present at the XXIV Congress of the International Society on Thrombosis and Haematosi in Amsterdam, Netherlands. The title of his talk was 'Platelets promote immunopathology in P. Berghei infection by inhibiting the development of IL-10 expressing Th1 cells'.



**Charlotte Mills** is a post graduate student supervised by Prof Jeremy Spencer. The travel award that she received was used to attend the International Conference on Polyphenols and Health (ICPH) in Buenos Aires, Argentina. At the conference she presented her work on coffee polyphenols and vascular function as a poster, and was further awarded an ICPH Travel Prize at the conference.



**Sarah Jing-Guo** is a PhD student supervised by Professors Ian Givens and Julie Lovegrove. She received the travel award for attending the Nutrition Society conference on 'Eggs-lifting the limits and establishing the nutritional benefits', which was held at the Royal Society of Medicine, London, UK (8 May 2013).



**Rekha Rana** is a post graduate student supervised by Prof Jon Gibbins. She received the travel award to attend and present at the XXIV Congress of the International Society on Thrombosis and Haematosi in Amsterdam, Netherlands. She gave an oral presentation on the topic 'Role of EphB2-Ephrin signalling in platelet function'.

**Francis Atanu** is a PhD student supervised by Prof Kim Watson. He received the ICMR Conference and Travel Award to attend and present a poster at the International Conference on Bioinformatics, Computational Biology and Biomedical Informatics held in Washington DC, USA (September 22–25 2013).

The other winners were **Jordi Mayneris-Perxachs**, **Diana Barker** and **Alejandro Giraldo Ramirez**.

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.



**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 email

[nutritionvolunteers@reading.ac.uk](mailto:nutritionvolunteers@reading.ac.uk)

**HIGHLIGHTS**

**i** For more information, please contact:

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