



**LEEDS INSTITUTE FOR GENETICS, HEALTH AND THERAPEUTICS**

**ANNUAL POSTGRADUATE RESEARCH STUDENT SYMPOSIUM**

**24<sup>th</sup> January 2012**

**Rooms MTC X & Y – Level 8 Worsley Building**

**Make a note in your diary for next year's Symposium**

**on Tuesday 26<sup>th</sup> March 2013**

**Programme: LIGHT Annual Postgraduate Research Symposium**  
**24<sup>th</sup> January 2012 (Worsley Level 8; Room MTC X & Y)**

- 9.30-9.50     **Tea/Coffee and Poster viewing** (Room MTC Y)
- 9.50-10.00   **Welcome**
- 10.00-11.00   **Oral Paper Session 1** (Room MTC X)
- 10.00-10.20   The Faculty of Medicine and Health's Researcher Development Programme – careers preparation for postgraduate researchers – *Emma Spary*
- 10.20-10.40   Identifying neighbourhood inequalities effect on heart attack recovery – *Elizabeth Metcalfe*
- 10.40-11.00   The effect of aflatoxin exposure on child growth and cancer development – *Jovina Castelino*
- 11.00-11.30   **Tea/Coffee and Poster viewing** (Room MTC Y)
- 11.30-12.30   **Oral Paper Session 2** (Room MTC X)
- 11.30-11.50   Circadian variation in vascular and metabolic indices in a mouse model of diet-induced obesity: divergence of insulin resistance and loss of molecular clock rhythm – *Honey Prasai*
- 11.50-12.10   Vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium – *Jayne Hutchinson*
- 12.10-12.30   Factors influencing whether or not new innovations are adopted in routine healthcare settings: case studies on the introduction of home telehealth – *Victor Joseph*
- 12.30-1.30    **Lunch for Registered Attendees and Poster viewing** (Room MTC Y)
- 1.30-3.00     **Oral Paper Session 3** (Room MTC X)
- 1.30-2.00     LIGHT Early Career Group: you asked, we've responded – *Matthew Gage and Sarah Fleming*
- 2.00-2.20     A method for identifying abnormal albumin levels using an MRI contrast agent – *Owen Richardson*
- 2.20-2.40     The influence of vitamin E bonded haemodialysis membranes on erythropoiesis stimulating agent requirements, fibrin clot structure and function, oxidative stress, inflammation and clinical outcomes – *Simon Lines*
- 2.40-3.00     Identification of genes and mechanisms that regulate oocyte maturation following gene knockdown by microinjection of sheep oocytes – *George Liperis*
- 3.00-5.00     **Tea/Coffee and Poster viewing** (Room MTC Y)
- 5.00-5.15     Presentation of Prizes
- 5.15-6.30     **Early Career Research Group Reception and Speed Networking**
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## Posters

1. Enhancing vascular endothelial repair in the setting of insulin resistance: effects of insulin-like growth factor binding protein-1 – *Amir Aziz*
2. Accuracy of Extend for hypofractionated stereotactic radiotherapy – *Béatrice Reiner*
3. Carcinogenic mycotoxin dietary exposure assessment in Tanzanian children – *Candida Shirima*
4. The relationship between agrobiodiversity, dietary diversity and nutrition in Sub Saharan Africa – *Cristina Cleghorn*
5. The vascular smooth muscle T-type calcium channel: an anti-proliferative target for heme oxygenase-1? – *Hayley Duckles*
6. Impact of stent type on survival following left main stem percutaneous coronary intervention – *Ian Pearson*
7. Molecular mechanisms behind differences in clot formation, lysis and morphology secondary to a mutation in the fibrinogen gene – *Katie Greenhalgh*
8. The optimisation of a two-step serum-free culture system enabling the development of ovine oocytes from early preantral follicles – *Lorna Blackwell*
9. High-resolution versus standard-resolution cardiovascular magnetic resonance myocardial perfusion imaging for the detection of coronary artery disease – *Manish Motwani*
10. Imputation and Survival Analysis: an example using cancer registry data – *Marlous van Laar*
11. Impact of early disability on six month physical function after stroke: a latent profile analysis – *Theresa Munyombwe*
12. Molecular mechanisms for the reduced clinical efficacy of aspirin in diabetes mellitus – *Zeyad Kurdee*
13. Assessment of Myocardial Perfusion with X-ray Angiography – *Andrew G Davies*

**Identifying neighbourhood inequalities effect on heart attack recovery – Elizabeth Metcalfe**

*Background and aims*

Within the UK the proportion of people reaching retirement age has been increasing for many years, sixty percent of heart attacks occur within people within this age range. There is a huge difference in mortality from heart attacks between locations across the country, this may be due to clinical factors such as treatment received, the hospital attended, the socioeconomic deprivation in which the person lives and recovers, or personal factors such as levels of social interactions, stressful environment such as crime rates and access to services such as cardiac rehabilitation, GP surgeries and public transport. Each of these elements are related in part to where the person lives.

*Methods*

Taking a mixed method approach using a combination of statistical approaches on a heart attack database of the Yorkshire area, and a small area study with in Yorkshire using questionnaires, with further work to include interviews and community mapping, I am examining the importance of the local neighbourhood on how well a person of retirement age recovers following a heart attack. By identifying which aspects of a neighbourhood have a positive or negative effect on recovery using a comparison of representative neighbourhoods, with an aim to identify the causes of inequalities.

*Initial results and conclusion*

Initial results of this progressive study show that quantitatively increased neighbourhood deprivation (determined by indicators IMD and Townsend) reduces survival, and qualitatively that participants perceive large open spaces and nice neighbourhoods, and that changes in diet and amount of exercise will increase recovery/decrease the chances of a second cardiac event.

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**The effect of aflatoxin exposure on child growth and cancer development – Jovina Castelino**

*Background and aims*

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) exposure through consumption of contaminated maize and groundnuts has been linked to liver cancer and impaired child growth in West Africa. The mechanisms for growth impairment are less well known than its carcinogenic effects. One hypothesis is that AFB<sub>1</sub> liver toxicity may affect the insulin-like growth factor (IGF) axis components such as IGF-1 and the IGF-binding proteins (IGFBPs), which play an important role in child growth. The aims of this study were to test this hypothesis and to understand the epigenetic effects of aflatoxin exposure on liver cell carcinogenesis.

*Methods*

Serum from 199 Kenyan school children were analysed for aflatoxin-albumin adduct (AF-alb), IGF-1 and IGFBP-3 levels using ELISA. HHL-16 'normal' liver cells were treated with 0.5 µg/ml, 5 µg/ml and 20 µg/ml AFB<sub>1</sub> for 48 hours. Gene expression of *IGF1*, *IGF2*, *IGFBP3* and *IGF1R* were analysed using qPCR. Additionally, to understand the effect of AFB<sub>1</sub> on tumourigenesis, DNA methylation was analysed by pyrosequencing of *IGF2*, *RASSF1a*, *MGMT*, *GSTP1*, *MLH1* and *p16* gene promoters in the AFB<sub>1</sub>-treated HHL-16 cells

*Results*

Children belonging to an exposure group of more than 400 pg/mg AF-alb were 5.9 cm shorter than children belonging to the lower exposure group, after adjusting for age ( $P=0.013$ ). AF-alb was also inversely associated with IGF-1 and IGFBP-3 levels ( $P=0.027$ ; 0.03). Path analysis suggested that the association between AF-alb and IGF-1 contributes ~10% of the total effect of AF-alb on child height.

The *in vitro* studies showed that there was reduction in expression of tested genes from cells treated with 5 µg/ml and 20 µg/ml AFB<sub>1</sub> with a dose-dependent effect being observed for *IGFBP3* and *IGF1R* genes, further supporting the current hypothesis. Pyrosequencing analysis revealed no major change in DNA methylation between treatments and controls.

#### *Conclusion*

This study indicates that aflatoxin exposure could be responsible for reduced child growth partially through reduced production of IGF proteins but further studies are required to determine whether epigenetic modifications of key genes contribute to the growth impairment and the carcinogenesis effect of AFB<sub>1</sub>.

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## **Circadian variation in vascular and metabolic indices in a mouse model of diet-induced obesity: divergence of insulin resistance and loss of molecular clock rhythm – Honey Prasai**

#### *Background and aims*

Circadian rhythms are integral to the normal functioning of numerous physiological processes and are controlled in peripheral tissues by the molecular clock, a negative feedback system of transcription factors which produces regular and repeating cycles of 24h length. Blood pressure, vascular endothelial activity and response to glucose and insulin challenge are all known to display robust circadian variation. There is also a rhythm of onset of adverse cardiovascular events such as myocardial infarction, which cluster in the early hours of the morning. Growing evidence indicates that this rhythm is lost in metabolic disease such as diabetes. Loss of circadian variation in blood pressure and systemic glucose homeostasis is also associated with diabetes and obesity. Obesity has been shown to impair the peripheral clock mechanism in liver and adipose but its effects in the cardiovascular system are not known. We investigated the effect of diet-induced obesity in a mouse model upon rhythmic molecular clock gene transcription and circadian variation in vascular and metabolic systems.

#### *Methods*

Male C57BL6J mice were fed either high fat diet or standard chow for 10 weeks. Circadian variation in vascular physiology was measured by ex vivo aortic vasomotion and by tail cuff blood pressure plethysmography. Circadian variation in metabolic homeostasis was measured by response to intraperitoneal challenge with glucose and insulin and by plasma insulin ELISA. Tissue events were interrogated further in aorta, liver and visceral adipose. Rhythmic transcription of the core clock genes *Bmal1* and *Per2* was measured by quantitative PCR (qPCR); clock-controlled insulin signalling was measured by protein immunoblotting for Akt and phosphorylated Akt; inflammation was measured by qPCR transcription of the macrophage marker *F4-80* and complement *C3*.

#### *Results*

In obese animals physiological rhythms were preserved in the vasculature but circadian variation in glucose tolerance ( $P<0.05$ ) and insulin sensitivity ( $P<0.01$ ) was blunted, which corresponded to disruption of rhythmic transcription of clock genes in adipose ( $P<0.001$ ) but preservation in aorta and liver. Impaired insulin signalling was found in aorta and liver ( $P<0.05$ ) but not in adipose. Expression of inflammatory markers was increased in obese adipose ( $P<0.001$ ) but not in other tissues.

#### *Conclusions*

We demonstrate that vascular tissues are less sensitive to pathological disruption of circadian rhythms during obesity than metabolic tissues and suggest that cellular disruption of clock gene rhythmicity may occur by mechanisms shared with inflammation but distinct from those leading to insulin resistance. This study emphasises that loss of circadian rhythm is an important finding in cardiovascular and metabolic disease and may be a neglected contributor to pathology.

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## **Vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium – Jayne Hutchinson**

### *Background and aims*

Vitamin C intake has been inversely associated with breast cancer risk in retrospective case-control studies, indicating a protective effect. However, meta-analyses of prospective cohort studies have not supported this. Food Frequency Questionnaires which are used to estimate intake in cohort studies can over-report fruit and vegetable intake, the main source of vitamin C. This current study is the first to investigate breast cancer risks in relation to vitamin C intake using recordings from food diaries, and the first to examine the relationship in UK women. It is also one of only a few studies to include intake from both supplements and diet in the analyses.

### *Methods*

Estimated dietary vitamin C intake was derived from four to seven days of food diaries and was pooled from five prospective studies in the UK Dietary Cohort Consortium. This nested case-control study of 851 incident breast cancer cases and 2727 matched controls examined breast cancer risk in relation to dietary vitamin C intake using conditional logistic regression adjusting for relevant covariates. Total vitamin C intake from supplements and diet was analysed in three cohorts.

### *Results*

No evidence of associations were observed between breast cancer risk and vitamin C intake analysed for dietary vitamin C intake (OR = 1.00 per 60mg/d, 95%CI: 0.91 to 1.09,  $P_{\text{trend}} = 1.0$ ) or total vitamin C intake (OR = 1.01 per 60mg/d, 95%CI: 1.00 to 1.03,  $P_{\text{trend}} = 0.1$ ). Additionally, there was no significant association for post-menopausal women for dietary (OR = 1.00 per 60mg/d, 95%CI: 0.89 to 1.12,  $P_{\text{trend}} = 0.9$ ) or total vitamin C intake (OR = 1.02 per 60mg/d, 95%CI: 1.00 to 1.05,  $P_{\text{trend}} = 0.06$ ).

### *Conclusions*

This pooled analysis of individual UK women found no evidence of significant associations between breast cancer incidence and dietary or total vitamin C intake derived uniquely from detailed diary recordings.

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## **Factors influencing whether or not new innovations are adopted in routine healthcare settings: case studies on the introduction of home telehealth – Victor Joseph**

### *Background and aims*

New innovations are increasingly being introduced into routine use in healthcare setting with varying degrees of their outcomes, yet little is understood why they are adopted or not. The aims of this study were to (1) describe the uptake of Telehealth in two phases of implementation; (2) determine the impact of Telehealth on hospital admissions; (3) determine the level of acceptance of Telehealth among patients and clinicians; and (4) explore possible reasons for the apparent differences in uptake of Telehealth in the two phases of Telehealth in Doncaster.

### *Methods*

A case-study research method was adopted, using *disciplined configuration* (employs established theory to explain a case) and *Theory Testing*. The study was guided by two principal theoretical frameworks: normalisation process model (NPM); and DEPOSE (design, equipment, procedures, operators, supplies and materials, and environment) model. Two main cases (studies) used were: (1) a randomised controlled trial (RCT); and (2) service evaluation; both involving Telehealth in Doncaster. Hospital admissions records were obtained from data in Doncaster Primary Care Trust (PCT) and quantitative analysis was conducted using STATA software and summary statistics, t-test and logistic regression modelling were performed. Qualitative interviews were carried out with patients and staff.

### *Results*

In the RCT, 36 patients were recruited (16 control group; and 20 intervention group). Logistic regression model to predict the likelihood of hospital admission showed adjusted odds ratio (OR) of 0.08 (p-value = 0.03) in favour of intervention (Telehealth) group. During service evaluation, 204 patients were referred and 176 used Telehealth. The average number of hospital admission per patient reduced from 2.18 (95% CI: 1.67, 2.69) before Telehealth to 1.20 (95% CI: 0.88, 1.52) during Telehealth period (p-value = 0.0004). The better uptake of Telehealth during service evaluation in comparison with that of RCT can be explained by a combination of factors including: change in service design, technological changes, different patient groups and better management of the project implementation. On the other hand, logistics and settings were not considered to be critical in determining the uptake of Telehealth in the two Phases of Telehealth implementation. Generally, patients on Telehealth expressed positive experiences about Telehealth and were generally satisfied with it. Staff, too were positive about Telehealth in enabling better communication, and picking problems early, although they acknowledged that it increased patients contacts and workload.

### *Conclusions*

The study shows that Telehealth is effective in reducing hospital admissions. Patients and staff gave positive experience about Telehealth. The interaction of factors related to service design, technology, patient group, and staff appear to explain the performance of Telehealth in routine healthcare setting.

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## **A method for identifying abnormal albumin levels using an MRI contrast agent – Owen Richardson**

### *Background*

Magnetic resonance imaging (MRI) uses the absorption and emission of energy by certain nuclei within an applied magnetic field to present a unique internal view of the body. MRI is used extensively in medical diagnosis, treatment planning and treatment response monitoring. Two relaxation rates (R1 and R2) characterise the return of 'excited' nuclei to their initial state through re-emission of absorbed energy; MRI utilises the variation in these relaxation rates to highlight the contrast between tissue types. Gadolinium-based contrast agents, injected into the patient, enhance image contrast by increasing the relaxation rates of nuclei in close proximity to the agent. Unlike other contrast agents, gadofosveset binds to albumin in the blood on injection, with binding fraction varying according to relative gadofosveset and serum albumin levels. Although binding increases gadofosveset effectiveness substantially at low magnetic fields, its influence on R1 is negligible at high field strengths. Conversely, bound gadofosveset R2 remains high regardless of field strength.

### *Aims*

It is suggested that pathology associated with an imbalance in intra/extravascular albumin distribution, such as trauma or sepsis, may be identified by monitoring levels of bound gadofosveset. The aims of this study were to assess whether:

- (1) At high magnetic fields, the overall gadofosveset concentration could be determined by the pre- to post-contrast increase in R1 ( $\Delta R1$ ), irrespective of albumin level;
- (2) The ratio of the pre- to post-contrast increases in R2 and R1 ( $\Delta R2/\Delta R1$ ) could provide a marker of albumin level, assuming the gadofosveset concentration calculated in (1).

### *Methods*

High-field MR images were obtained for in vitro solutions containing physiologically relevant levels of gadofosveset and serum albumin, and two-parameter mono-exponential model fits applied to measured signal intensity data to determine R1 and R2 values.

### Results

A strong linear relationship was shown between  $\Delta R1$  and gadofosveset concentration, with no influence from albumin level. Utilising this gadofosveset concentration and measured  $\Delta R2/\Delta R1$  value, relative albumin levels could be determined with reasonable accuracy. However, model accuracy deteriorated when albumin level exceeded gadofosveset concentration.

### Conclusion

Results suggest the process outlined above may be a viable method for identifying elevated or reduced protein levels in vivo, particularly where gadofosveset concentration exceeds albumin level.

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## **The influence of vitamin E bonded haemodialysis membranes on erythropoiesis stimulating agent requirements, fibrin clot structure and function, oxidative stress, inflammation and clinical outcomes – Simon Lines**

### *Background and Aims*

Haemodialysis (HD) patients have high rates of cardiovascular disease. Increases in oxidative stress, inflammation, high doses of erythropoiesis stimulating agents (ESAs) and a prothrombotic phenotype are all possible contributors. Vitamin E (VE)-bonded dialysis membranes have been developed and are purported to have favourable effects on a number of these parameters. The aims of this study were to carry out a 12-month randomised controlled trial to examine the effects of dialysing prevalent HD patients with a VE-bonded membrane on ESA requirements, oxidative stress, inflammation, fibrin clot phenotype, cardiovascular event rates, hospital admissions and mortality.

### *Methods*

All Leeds Teaching Hospitals Trust HD patients were screened for study inclusion; principle exclusion criteria were being on HD < 3 months or significant inflammation at baseline (defined as C-reactive protein > 50 mg/L). Patients were randomised to HD with a VE-bonded membrane or a non-VE-bonded equivalent. All ESA dosing was performed by means of a computer based anaemia management algorithm. Patients underwent blood sampling for oxidatively-modified low-density lipoprotein, CRP, complement C3 and fibrin clot structure analysis at baseline, 6 and 12 months. Data were collected on monthly haemoglobin levels and ESA dose, admissions to hospital, cardiovascular events and mortality.

### *Results*

Of the 500 HD patients screened, 260 were enrolled in the study and 123 assigned to the VE group. Both groups were well matched at baseline with the exception of more diabetics in the VE group (35% vs 23%;  $p=0.03$ ). Analysis of haemoglobin and ESA data revealed no significant inter-group differences or changes over time in either group. Post hoc analysis by tertiles of ESA dose at baseline revealed significantly lower ESA doses at 12 months for the highest tertile of patients dialysing with the VE membrane (0.45 [0.29-0.71] vs 0.54 [0.42-0.68] mcg/kg/wk;  $p=0.02$ ) with no significant change in the control group ( $p=0.81$ ). Analysis of the fibrin clot parameters revealed no effect of the VE-membrane on clot density or fibrinolysis times. The key findings regarding the determinants of fibrin clot structure were: (i) patients with ischaemic heart disease (IHD) formed denser clots which were less susceptible to fibrinolysis, (ii) diabetic status did not influence clot density or fibrinolysis although fibrinolysis times were positively correlated with HbA1c levels and (iii) both clot density and fibrinolysis times were positively correlated with CRP and C3 levels. The CRP levels changed significantly over time ( $p<0.001$ ) but there was no effect of treatment group ( $p=0.24$ ) or significant interaction of treatment group and time ( $p=0.37$ ). The C3 levels decreased over time in both groups ( $p<0.001$ ) with no inter-group differences in terms of absolute values ( $p=0.38$ ) or the pattern of change ( $p=0.67$ ). Hospital admissions, mortality and cardiovascular events did not differ between groups.

### *Conclusion*

HD with a VE membrane may reduce ESA requirements in patients requiring larger doses. No effects of VE on inflammation or clot phenotype were observed. The findings of altered clot phenotype in HD patients with IHD but not diabetes merits further investigation, particularly with respect to cardiovascular risk.

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## **Identification of genes and mechanisms that regulate oocyte maturation following gene knockdown by microinjection of sheep oocytes – *George Liperis***

### *Background and aims*

The development of methods for growing oocytes to maturity *in vitro* and characterisation of eggs capable of being fertilised encompasses many attractions for a variety of fields including human fertility treatment, animal production, conservation of endangered species and research. In order to understand how oocytes grow and attain developmental competence, genes and mechanisms that regulate these processes must be identified. RNA interference (RNAi) can be used as a tool in order to determine functional roles for genes during specific periods of development, such as oocyte maturation. The aim of this project was to utilise RNAi in conjunction with oocyte cultures in order to identify the function of genes in oocyte maturation.

### *Methods*

Application of short interference RNA (siRNA) through microinjection on sheep germinal vesicle (GV) stage oocytes was used in order to create a gene specific knockdown. Following a two day culture and determination of the knockdown, the function of the gene in question was determined by both molecular and morphological evaluations. Gene knockdown was originally applied for Growth differentiation factor 9 (GDF9), an oocyte derived factor with an established role in oocyte maturation followed by application of siRNA for Gametocyte specific factor 1 (GTSF1), a gene with an unknown role in oocyte maturation. The function of the later gene was examined because of its gamete specific pattern of expression including a highly conserved sequence capable of binding RNA and potentially important for preservation of fertility.

### *Results*

Following a two day culture, successfully knockdowned GDF9 oocytes had a reduced ability to mature to the Meiosis II stage (MII) as well as reduced level of cumulus expansion, both being parameters of developmental competence. Molecular evaluations by real time PCR confirmed the knockdown of the gene additionally to a significant reduction in the mRNA levels of Hyaluronan synthase 2 (*HAS2*), a gene responsible for driving cumulus expansion. Confirmation of the GDF9 function and the successful application of siRNA microinjection technology continued with examination of GTSF1. Following characterisation of its expression in the ovine ovary, *GTSF1* was successfully knockdowned at the oocyte level. Although morphological evaluations did not indicate significant effects on developmental competence, ongoing microarray analysis comparison between knockdown and control oocytes, have the prospective of providing noteworthy insights into the function of this gene in oocyte maturation.

### *Conclusion*

Successful knockdown of genes during well defined periods of development can result in the identification of genes that regulate oocyte development. Further research and identification of these genes can have a major impact in the field of assisted conception and fertility preservation.

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## Abstracts of Poster Presentations (in order of presentation)

### 1. Enhancing vascular endothelial repair in the setting of insulin resistance: effects of insulin-like growth factor binding protein-1 – Amir Aziz

#### *Background and aims*

Insulin resistance is a key feature of type 2 diabetes and predisposes to cardiovascular disease by inducing endothelial dysfunction. Damage to the biochemical and/or structural integrity of endothelial cells arises as a consequence of exposure to risk factors and revascularisation procedures, leading to adverse remodelling and atherosclerosis. In health, endogenous repair mechanisms are in place to regenerate injured endothelium. However, our laboratory has recently shown that insulin resistance impairs the capacity for endothelial repair by reducing the mobilisation and functionality of circulating endothelial progenitor cells. Additionally, we have discovered that a circulating protein, insulin-like growth factor binding protein-1 (IGFBP-1), is potentially protective in the vasculature by stimulating nitric oxide production and enhancing insulin sensitivity. In cross-sectional studies, low IGFBP-1 levels are associated with diabetes and cardiovascular disease. IGFBP-1, a 30kDa protein produced predominantly in the liver, is responsible for short-term modulation of insulin-like growth factor (IGF) bioactivity but can also modulate migration and proliferation of certain cell types independently of IGF. In this project, I am investigating whether IGFBP-1 can enhance vascular endothelial repair in insulin resistant mice *in vivo* and dissecting the potential mechanisms by examining the effects of IGFBP-1 on human endothelial cells and endothelial progenitor cells *in vitro*.

#### *Methods*

To assess endothelial regeneration, control and insulin receptor (IR)<sup>+/-</sup> mice with and without transgenic over-expression of IGFBP-1 (IGFBP1tg) underwent femoral artery endothelial denuding wire-injury. After five days, mice were anaesthetized and 5% Evans Blue dye was injected. The area of regenerated endothelium was quantified in *en face* sections of injured artery. The effects of IGFBP-1 on the functional properties of endothelial cells *in vitro* were examined using modified Boyden chamber and scratch wound healing assays.

#### *Results*

Endothelial regeneration was unchanged in IGFBP1tg mice compared to control animals. Endothelial regeneration was blunted in IR<sup>+/-</sup> mice, but with a strong trend to restored regeneration in this model of insulin resistance observed in mice overexpressing IGFBP-1 (re-endothelialised area 46+/-1% vs. 54+/-3% at day 5 following injury, p value 0.09). In human umbilical vein endothelial cells (HUVEC), the pro-inflammatory cytokine tumor necrosis factor-alpha significantly inhibited migration in the scratch wound assay (P < 0.01). Co-incubation with IGFBP-1 restored the migratory capacity of HUVEC to control levels. There was a trend to enhanced migration of Human Coronary Artery Endothelial Cells (HCAEC) in response to IGFBP1 in the Boyden chamber assay (P=0.07).

#### *Conclusion*

IGFBP-1 over-expression has the potential to enhance endothelial repair in the insulin resistant setting. Experiments are ongoing to confirm these findings and to elucidate the time course of the pro-regenerative effects. In HUVEC, IGFBP-1 restores cell migration in a pro-inflammatory setting. Further experiments will allow me to determine the molecular basis of this modulatory effect and to investigate whether a similar effect of IGFBP-1 on endothelial progenitor cells complements the effects on mature endothelial cells. Collectively, these studies will determine whether IGFBP-1 could be a target to prevent vascular disease in insulin resistance.

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## 2. Accuracy of Extend for hypofractionated stereotactic radiotherapy – *Béatrice Reiner*

### *Introduction*

Gamma Knife stereotactic radiosurgery (SRS) is an efficient way to treat brain lesions. The high accuracy is achieved with the G frame, a system that is screwed directly to the skull bone. The current limitations of Gamma Knife SRS treatments are target size and organs at risk (OAR). To overcome these limitations a non-invasive relocatable system, known as Extend, has been developed. The key feature is a moulded mouth piece held in place by vacuum suction that stops the treatment when the patient moves out of the ideal position.

### *Material and Method*

Six volunteers were set-up five times and the reposition accuracy was analysed. The setup accuracy is verified with the Reposition Check Tool (RCT) which measures the distance from reference positions to the patient surface from four directions and calculates the total shift vector. The total vector was analysed to evaluate the achievable accuracy while the individual points were analysed to evaluate the origin of any deviation. The sensitivity of the vacuum surveillance is measured using a range of dental models. A special holder has been created to move the model in small steps (0.1mm per step) out of position to evaluate the trigger level (work in progress).

### *Results*

Repositioning within 1mm was achieved in 29 out of 30 set-ups, with 87% (26/30) within 0.6mm. Reproducibility improved over time for 5 out of 6 patient measurement series. Evaluating the individual points, especially the lateral shift, resulted in a measurement uncertainty of 0.3mm (range -0.8 to 0.8mm) at a level of 0.3mm (1SD).

### *Conclusion*

Repositioning with Extend is accurate and reliable. Set up deviations can be minimised if the setup is practiced 2-3 times before the CT scan is done. With such accuracy Extend enables hypofractionated treatment to reduce the risk to OAR's, especially optic nerve and chiasm.

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## 3. Carcinogenic mycotoxin dietary exposure assessment in Tanzanian children – *Candida Shirima*

### *Background and aims*

Aflatoxins and fumonisins are frequent maize contaminants. Maize is staple food and main ingredient of weaning food for children in most of regions in Tanzania, justifying high risk of exposure to aflatoxins and fumonisins. The collaborative project between University of Leeds and Tanzania aims to utilize exposure biomarkers to evaluate the exposure of young children to aflatoxins and fumonisins through maize based weaning foods in three regions of different agro-ecological zones in Tanzania namely Iringa, Kilimanjaro and Tabora.

### *Methods*

162 children aged 5 to 14 months from one village in each of the above three regions of Tanzania were recruited upon maize harvest season in 2010. Blood sample were collected intravenously for aflatoxin albumin adduct (AF-alb) biomarker analysis using ELISA technique. Morning urine was collected using a paediatric urine bag for urinary fumonisin B1 (FB1) biomarker analysis using LC-MS method. Body weight and length were measured according to standard method and weigh for age (WAZ), length for age (LAZ) and weight for length (WLZ) Z-scores were calculated based on WHO criteria. Child information on age, sex, weaning and breast feeding practice, as well as family social economic status (SES) were also collected. Statistical analysis was performed using STATA.

### *Results*

Preliminary data analysis results demonstrate that children are highly exposed to aflatoxins and fumonisins. AF-alb geometric mean level (95% Confident Interval (CI)) is 5.0 (4.2, 6.0) while the mean level of urine FB1 (95% CI) is 312.8 (255.8, 382.4). Highest aflatoxin and fumonisin exposures were found in the village of Tabora. Prevalence of stunting, underweight and wasting among the studied children was 41%, 8% and 3% respectively. The village of Iringa has the highest stunting prevalence at 55%.

### *Conclusion*

These findings demonstrate that children in Tanzania are exposed to both aflatoxin and fumonisin from diet following weaning, which may potentially contribute to the high stunting prevalence. Further investigation is required to understand the contributing factors for the high stunting prevalence. Furthermore, intervention measures to reduce aflatoxins and fumonisins exposure need to be implemented in order to prevent the health risks associated with early life exposure to mycotoxins.

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## **4. The relationship between agrobiodiversity, dietary diversity and nutrition in Sub Saharan Africa – Cristina Cleghorn**

### *Background*

There are simultaneously significant pressures on the global environment while approximately two billion people are malnourished (Foresight report 2011). To reach two of the eight millennium goals ([www.un.org/millenniumgoals/](http://www.un.org/millenniumgoals/)) solutions to global hunger need to be reached sustainably and an increased understanding of how these issues are linked is essential.

### *Aim*

To investigate the relationship between agrobiodiversity, dietary diversity and nutrition in Sub-Saharan Africa.

### *Methods*

Nationally representative data about diet, nutrition and household demographics from the 1999 Tanzanian Demographic and Health Survey (DHS) will be linked with land cover data collected in 2000 by the GLC2000 project. These analyses will be repeated with Tanzanian DHS data from 2010 and land cover data from GlobCover2009. These results will be integrated and analysed using the spatial analysis software ArcGIS. These analyses will allow broad conclusions about the relationship between land cover and dietary diversity and nutrition outcomes to be made. The results of these analyses will also be used to select appropriate villages in Tanzania for the primary data collection to take place. The relationships between agrobiodiversity, dietary diversity and nutrition will be explored using data collected in at least two communities in rural Tanzania. Ecological methods will be used to assess agrobiodiversity and a questionnaire based household interview will be used to collect dietary diversity, food access, demographic and livelihoods data. Heights, weights and mid upper arm circumference of the household members will be measured directly.

### *Progress to date*

The questionnaire and ecological methods have been designed based on available literature. These methods were piloted in April 2011 in Shebomeza, a small rural community in the district of Muheza which lies in the north of Tanzania. Methods have subsequently been redesigned in preparation for the primary data collection in Tanzania in 2012. DHS data analysis is in its early stages but basic nutrition and land cover data have been combined and clusters with high prevalence of stunting, wasting and underweight have been identified.

### *Conclusions*

This research has the potential to provide rich data that may provide insights into the environmental and social determinants of nutritional status in Sub-Saharan Africa. The findings of this research will be widely shared to help inform future research as well as policy to improve health in Sub-Saharan Africa.

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## **5. The vascular smooth muscle T-type calcium channel: an anti-proliferative target for heme oxygenase-1? – Hayley Duckles**

### *Background and aims*

Excess proliferation of vascular smooth muscle cells (VSMC) is a central feature of vascular disorders such as hypertension, restenosis and atherosclerosis (1). T-type calcium channels provide an important route for calcium entry in disease-associated VSMC proliferation, and under these conditions VSMC also increase expression of heme oxygenase-1 (HO-1) (1) (2). HO-1 catabolises free heme to produce biliverdin, iron, and carbon monoxide (CO), and exhibits anti-proliferative effects, possibly through the production of CO (2). We aimed to investigate the effects and possible interactions of HO-1, CO, and T-type calcium channel inhibition on VSMC proliferation.

### *Methods*

VSMC were explanted from fragments of saphenous vein (SV) of patients undergoing coronary bypass and propagated in primary culture. Proliferation assays were performed over 4 days in the absence or presence of the T-type channel inhibitor mibefradil, an inducer of HO-1, CoPPIX, or the CO donor compound, CORM-3. Viable cells were counted using trypan blue and a hemocytometer. Statistical analysis was performed using two-tailed paired T-test and one-way ANOVA with Bonferroni's multiple comparison test, as appropriate.

### *Results*

Proliferation was reduced by  $39.7 \pm 8.8\%$ ,  $p < 0.05$ ,  $n=3$ , in the presence of  $3\mu\text{M}$  mibefradil, and by  $37.7 \pm 2.8\%$ ,  $p < 0.05$ ,  $n=3$ , following HO-1 induction by  $3\mu\text{M}$  CoPPIX (induction verified by western blotting,  $n=2$ ). CORM-3 application ( $3\text{--}30\mu\text{M}$ ) produced a dose dependent decrease in proliferation, a reduction of  $36.0 \pm 4.4\%$ ,  $P < 0.01$ ,  $n=3$ , was observed in the presence of  $3\mu\text{M}$  CORM-3. The application of  $3\mu\text{M}$  mibefradil and  $3\mu\text{M}$  CoPPIX simultaneously reduced proliferation by  $57.7 \pm 8.1\%$ ,  $P < 0.05$ ,  $n=3$ .

### *Conclusions*

These preliminary data support the idea that CO is antiproliferative through inhibition of VSMC T-type calcium channels. This signalling pathway may therefore be a novel target for pharmacological intervention in vascular disorders involving excess SMC proliferation.

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## 6. Impact of stent type on survival following left main stem percutaneous coronary intervention – Ian Pearson

### *Background and aims*

There are conflicting data on survival after left main stem (LMS) percutaneous coronary intervention (PCI) by stent type. The aim of this study was to compare survival between patients treated with drug eluting stents (DES) and bare metal stents (BMS).

### *Methods*

We recorded the baseline characteristics of 268 consecutive patients undergoing LMS PCI at a single centre from 09/08/11 - 19/04/10. Mortality data was provided by the Office of National Statistics.

### *Results*

Mean (SD) age: 70.2 (12.8) years, 194 (72.4%) males. Diabetes: 18.6%; previous PCI: 15.3%; protected LMS: 20.4%. ST elevation myocardial infarction (STEMI): 9.7%, non ST elevation myocardial infarction (NSTEMI): 56.0%, chronic stable angina (CSA): 34.3%. Mean (SD) SYNTAX score: 36.7 (14.5), median EuroSCORE: 9.1. DES: 65.7% of cases. There were 74 deaths over 602 patient years (maximum 5.1, median 1.8). Mortality at 5.1 years was 27.6%. There were survival differences when data were stratified by BMS versus DES. The table shows the 95% HR for the cohort.

	Censored at 30 days.	Censored at 1 year.	Full survival:5.6 years
Unadjusted	0.28 to 1.56	0.10 to 0.35	0.33 to 0.82
Model 1	0.31 to 2.07	0.13 to 0.50	0.36 to 0.91
Model 2	0.31 to 1.84	0.10 to 0.36	0.38 to 0.96
Model 3	0.31 to 2.08	0.11 to 0.46	0.40 to 1.03
Model 4	0.22 to 1.10	0.09 to 0.35	0.36 to 0.90
Model 5	0.35 to 2.26	0.10 to 0.44	0.42 to 1.06

Model 1: Clinical presentation: STEMI, NSTEMI, CSA. Model 2: EuroSCORE. Model 3: Model 1 + model 2. Model 4: SYNTAX score. Model 5: Model 3 + model 4.

### *Conclusions*

Survival following LMS PCI, both unadjusted and adjusted for clinical characteristics, was greater when patients received a DES than a BMS at 1 year. No significant difference was seen at 30 days or at long-term follow-up.

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## 7. Molecular mechanisms behind differences in clot formation, lysis and morphology secondary to a mutation in the fibrinogen gene – Katie Greenhalgh

### *Background and aims*

The blood clot is composed of a mesh of fibrin fibres with cellular elements embedded in this network. The structure of the fibrin clot can determine predisposition to atherothrombotic conditions, as compact clots composed of thin fibres and small pores are associated with premature and more severe cardiovascular disease (CVD). The fibrinogen B $\beta$ Arg448Lys mutation is associated with changes in fibrin clot formation, structure and lysis implicating it in increased risk of CVD. Impairment in lateral aggregation, altered FXIII cross-linking and differences in interactions with plasma proteins are possible causes resulting in distinct structural and functional properties of clots formed from Arg448 and Lys448 fibrinogen. The aim of this work is to investigate the molecular mechanisms behind differences in clot formation, lysis and morphology secondary to mutations in the fibrinogen gene by analysing the effects of Arg448Lys mutation on early protofibril formation, FXIII activation and cross-linking as well as binding and cross-linking of  $\alpha$ 2-AP to fibrinogen.

### Methods

Clot structure and lysis were investigated using recombinant protein purified by affinity chromatography with the use of turbidity assays and confocal microscopy. The effects of Arg448Lys mutation on FXIII activation and cross-linking were investigated as well as the potential differences in binding and crosslinking of  $\alpha_2$ -AP to the variants of fibrinogen by binding ELISA, crosslinking SDS-PAGE gels and immuno-assay.

### Results

B $\beta$ 448Lys variant has significantly lower turbidity than B $\beta$ 448Arg ( $0.016 \pm 0.00097$  and  $0.0905 \pm 0.00201$  respectively,  $p < 0.05$ ). Time to full lysis (LT) was not significantly different between B $\beta$ 448Arg and B $\beta$ 448Lys in the absence of  $\alpha_2$ -AP (32.2 minutes  $\pm$  2.4 and 32.2 minutes  $\pm$  1.8 respectively). However,  $\alpha_2$ -AP significantly increased LT in both fibrinogen variants with an effect that was more pronounced in B $\beta$ 448Lys variant (3.2 minutes and 22.8 minutes  $p < 0.05$ ).  $\alpha_2$ -AP prolonged time to half lysis ( $t_{1/2}$ ) in both variants, proportional to the concentration of  $\alpha_2$ -AP in the presence of FXIII. The increase in LT elicited by  $\alpha_2$ -AP in the presence of FXIII was significantly greater than in its absence (reduction of 22% and 207% in 448Arg and 448Lys respectively,  $p < 0.05$ , following removal of FXIII).  $\alpha_2$ -AP increased LT significantly more in B $\beta$ 448Lys variant at every concentration. Preliminary studies suggest there is no difference in the rate or overall activation of FXIII between B $\beta$ Arg448Lys variants or any significant difference between rate or overall crosslinking of  $\alpha_2$ -AP into the clot.

### Conclusion

The mechanisms behind the observed prolongation in lysis are unclear. The observation that FXIII plays a role in  $\alpha_2$ -APs inhibitory effect on fibrinolysis indicates that cross-linking, rather than binding, of the protein plays a key role in  $\alpha_2$ -AP induced changes in lysis. On the other hand, the inhibition of fibrinolysis in B $\beta$ 448Lys compared to the B $\beta$ 448Arg in the absence of FXIII suggests that binding also plays a role. The lack of significant difference between the rate and overall binding and crosslinking of FXIII and  $\alpha_2$ -AP to the two variants despite the marked difference in prolongation of lysis by these agents suggests differences in structure of the fully formed fibrils and final clot morphology between the variants accounts for the observed results.

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## 8. The optimisation of a two-step serum-free culture system enabling the development of ovine oocytes from early preantral follicles – Lorna Blackwell

### Background and Aims

The development of techniques enabling the *in vitro* growth (IVG) and maturation of human oocytes would be highly beneficial in the field of fertility preservation. Previous research suggests that a multi-step culture system is optimal for the IVG of oocytes as the requirements of the developing oocyte and follicle are constantly changing. The aim of this investigation was to optimise the first stage of IVG with respect to culture length and the method by which the tissue was processed prior to culture.

### Method

Ovine ovarian follicles were cultured *in situ* within thin slices ( $\sim 0.5$  mm) of cortical tissue, in serum-free medium. Follicles were cultured for a period of either 6 days (short-term culture) or 16-23 days (long-term culture). In the initial investigations follicles at a later developmental stage than early primary were excised from the tissue. However, in subsequent short-term cultures primary and transitional follicles were not excised. Two methods of tissue dissection were utilised in which tissue was either cut into strips and teased apart using needles to remove the majority of cortical and stromal tissue or into squares ( $2 \text{ mm}^2$ ). At the end of the culture period preantral follicles were dissected using needles. Partial disaggregation of the tissue with the use of proteolytic enzymes was necessary after short-term, but not long-term culture as the tissue had softened enough to allow mechanical isolation alone to be utilised. If sufficient follicles were obtained these were put into isolated culture and the growth and survival rates analysed. The viability of the tissue and follicles were analysed using neutral red staining.

### *Results*

After long-term culture of ovine ovarian cortical tissue containing only primordial and early primary follicles it is possible to isolate secondary follicles. These follicles could then be cultured individually and were capable of further growth and development up to the early antral stage. Short-term culture did not yield secondary follicles unless the cultured tissue contained primary and transitional follicles from day zero. Survival and growth rates of isolated follicles were dependent upon their size at the time of isolation; optimal rates were achieved with follicles 180  $\mu$ m in diameter or larger. The use of neutral red staining revealed that the method of dissection in which the tissue was teased apart reduced the viability of the cortical tissue.

### *Conclusion*

It is possible for ovine ovarian follicles to grow and develop from the early primary to the early antral stage using a two-step culture system. Six days is not a sufficient length of time for follicles to develop from the early primary to the secondary stage. The method of tissue dissection does not appear to affect follicle yield, however, excessive handling of the tissue with needles detrimentally affects its long-term viability.

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## **9. High-resolution versus standard-resolution cardiovascular magnetic resonance myocardial perfusion imaging for the detection of coronary artery disease – Manish Motwani**

### *Background and Aims*

Although accelerated high-spatial-resolution cardiovascular magnetic resonance (CMR) myocardial perfusion imaging has been shown to be clinically feasible, there has not yet been a direct comparison with standard-resolution methods. We hypothesised that higher spatial resolution detects more subendocardial ischemia and leads to greater diagnostic accuracy for the detection coronary artery disease (CAD). This study compared the diagnostic accuracy of high-resolution and standard-resolution perfusion-CMR in patients with suspected CAD.

### *Methods*

One-hundred and eleven patients were recruited to undergo two separate perfusion-CMR studies at 1.5-T, one with standard-resolution (2.5 x 2.5mm in-plane) and one with high-resolution (1.6 x 1.6 mm in-plane) acquisition. High-resolution acquisition was facilitated by eight-fold *k-t* broad linear speed up technique (BLAST) acceleration. Two observers visually graded perfusion in each myocardial segment on a 4-point scale. Segmental scores were summed to produce a perfusion score for each patient. All patients underwent invasive coronary angiography and CAD was defined as stenosis  $\geq$  50% luminal diameter (quantitative coronary angiography).

### *Results*

CMR data were successfully obtained in 100 patients. In patients with CAD (n=70), more segments were determined to have subendocardial ischemia with high-resolution than with standard-resolution acquisition (279 vs.108;  $p < 0.001$ ). High-resolution acquisition had a greater diagnostic accuracy than standard-resolution for identifying single-vessel disease (area under the curve [AUC]: 0.88 vs. 0.73;  $p < 0.001$ ) or multi-vessel disease (AUC: 0.98 vs. 0.91;  $p = 0.002$ ) and overall (AUC: 0.93 vs. 0.83;  $p < 0.001$ ).

### *Conclusions*

High-resolution perfusion-CMR has greater diagnostic accuracy than standard-resolution acquisition for the detection of CAD in both single and multi-vessel disease and detects more subendocardial ischemia.

## 10. Imputation and Survival Analysis: an example using cancer registry data – *Marlous van Laar*

### *Introduction*

Stage of disease at presentation of cancer is not routinely recorded in medical records, resulting in large amounts of missing data. We investigated survival trends by ethnicity amongst 0-29 year olds with cancer whilst using multiple imputation (MI) to impute missing values of stage.

### *Method*

Subjects (n=3534) diagnosed with cancer in Yorkshire (1990-2005) were analysed. Individual imputation models were used to assign stage of disease to four main diagnostic groups; leukaemia, lymphoma, central nervous system (CNS) and other solid tumours. Linear regression was used to impute white blood cell count (WCC) for leukaemia as a proxy for stage and ordinal logistic regression was used to impute for the remaining diagnostic groups. The survival analysis was performed using Cox regression.

### *Results*

Missing stage data occurred in 66% of cases for lymphoma, 28% for CNS tumours, and 69% for other solid tumours. WCC was missing for 57% of leukaemia cases. Results of the final analysis showed an increased risk of death for south Asians compared to non-south Asians with leukaemia (HR=1.61; 95%CI=1.01-2.55) and lymphoma (HR=2.05; 95%CI=1.09-3.87), and a decreased risk for south Asians with other solid tumours (HR=0.50; 95%CI=0.28-0.89). There was no significant difference by ethnic group for those with CNS tumours (HR=1.51; 95%CI=0.82-2.78).

### *Conclusion*

Although stage was missing in two-thirds of cases overall, MI was used to minimise bias and enhance the precision of analyses. This technique therefore offers considerable advantages over other approaches such as complete case analysis or coding missing data as a separate category.

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## 11. Impact of early disability on six month physical function after stroke: a latent profile analysis – *Theresa Munyombwe*

### *Introduction*

Assessment of initial post-stroke disability is essential to guide development of patient centred rehabilitation goals. Latent profile analysis (LPA) is a statistical method that defines homogenous subgroup classifications according to patient characteristics (classes). These classifications may be useful to clinicians for treatment planning. We used LPA to classify patients according to multiple baseline stroke disability measures and examined their relationship with six month functional outcome.

### *Methods*

312 patients recruited into a prospective cohort study from three stroke units were included. Process, case-mix and stroke severity variables) were collected at baseline (Barthel Index (BI), Nottingham Extended Activities of Daily Living (NEADL) and GHQ\_12. NEADL was collected at six months. Latent classes were formed on BI, GHQ-12 and the four NEADL domains. Age, sex, pre-stroke disability, living alone and urinary incontinence were included in a single step regression model to form the classes. Latent classes were validated through multinomial regression modelling. The effect of these classes on six month NEADL was explored through a multivariable regression model (using outcomes data from 165 patients).

### *Results*

A three class solution was chosen, labelled mild, moderate and severe. Baseline BI and NEADL subscores increased, and GHQ\_12 decreased from class 1 (severe) to class 3 (mild). Compared with

patients in the mild class, patients in the severe class were 3.8 times more likely to have had a previous stroke and less likely to have been independent prior to stroke. Adjusting for care processes, patients in the mild class had significantly higher six month NEADL scores than patients in the severe class.

### *Conclusions*

The LPA was informative in defining three baseline stroke severity typologies. Baseline class membership is associated with functional outcome at six months. The characteristics of patients in the emerging classes can inform patient management. The classifications may also be used for stratifying patients or for case-mix adjustment.

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## **12. Molecular mechanisms for the reduced clinical efficacy of aspirin in diabetes mellitus – Zeyad Kurdee**

### *Introduction*

Diabetes is associated with higher risk of vascular occlusive disease. One mechanism is related to the associated prothrombotic environment secondary to enhanced platelet activation, increased levels/activity of thrombotic coagulation factors and impaired fibrinolysis. Aspirin remains the first line antiplatelet therapy used for primary and secondary prevention of cardiovascular disease. However, recent evidence indicates the clinical efficacy of aspirin is reduced in diabetes by mechanisms that are not fully understood.

### *Hypothesis and aims*

Aspirin treatment failure in diabetes is due to high plasma glucose affecting aspirin target and interfering with the therapeutic action of this agent. The aim of my work is to investigate the molecular mechanisms for the reduced clinical efficacy of aspirin in diabetes.

### *Methods*

Response of platelets to *ex vivo* and *in vivo* aspirin therapy was investigated using samples from diabetes subjects and matched healthy controls. The effects of aspirin on fibrin clot structure and fibrinolysis were also analysed, together with the role of this agent in platelet-fibrinogen interactions. Standard platelet aggregation tests are used to assess platelet reactivity, whereas turbidity curves, confocal and electron microscopy are employed to study clot structure/fibrinolysis.

### *Results*

Platelet aggregation tests have been optimised using light transmission aggregometry (LTA) and multiplate assays. Low concentration of *ex vivo* aspirin, as low as 0.01mg/l, reduced response to the platelet agonist arachidonic acid (AA) using LTA, while higher concentrations were needed in the Multiplate assay, probably secondary to the use of platelets only in LTA and whole blood in Multiplate test. Results from studying platelet aggregation response to AA, ADP and collagen from 13 healthy controls and 24 diabetes subjects, showed differences in response to aspirin treatment. Low concentration of aspirin (1mg/l), added *ex vivo*, resulted in reduced platelet aggregation response to AA in both patients and control. However, this inhibition was not seen in the presence of excess glucose (20 mmol/l) and significant inhibition of platelet activation was observed with 10 mg/l aspirin. As platelet aggregation in response to collagen can be partially affected by thromboxane production, high dose aspirin (10mg/l) suppressed platelet aggregation response to this agonist in both patients and controls, whereas low dose aspirin had no effect. Excess glucose had no effect on aspirin-induced inhibition on platelet activation following collagen stimulation. Aspirin had no effect on ADP-induced platelet stimulation in patients or controls in the absence or presence of additional glucose. Preliminary results of *in vivo* aspirin therapy (75 mg daily) in 16 diabetes subjects and 7 controls showed differences in the inhibitory effect of aspirin on platelet aggregation in response to AA and collagen. Differences between patients and controls were also observed with the clot structure response to *ex vivo* aspirin treatment.

### *Conclusion*

My preliminary data demonstrate possible differences between diabetes subjects and controls in relation to response to aspirin therapy. Confirmation of these data and further analysis of the pathways involved may help to understand the mechanisms for the reduced clinical efficacy of aspirin in diabetes.

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## **13. Assessment of Myocardial Perfusion with X-ray Angiography – Andrew G Davies**

### *Background*

X-ray angiography plays an important role in the diagnosis and treatment of coronary heart disease. Iodine based contrast medium is injected into the coronary arteries in order to assess blood flow in the heart in a series of x-ray images. Current x-ray angiographic practice focuses on the visual inspection of the morphology of the coronary arteries. Blood flow in the coronary arteries, however, is only a partial representation of the transit of blood through the heart- inadequate perfusion of blood into the myocardium is the cause of conditions ranging from stable angina through to Acute Coronary Syndrome. There is evidence that patency of the coronary arteries is not always a good indicator of adequate myocardial perfusion.

### *Aims*

To develop quantitative methods of assessing myocardial perfusion using x-ray angiography, and to optimise the radiographic imaging conditions to minimise radiation dose to the patient and staff due to such an investigation.

### *Materials and Methods*

Modifications have been made to an x-ray catheterisation laboratory to include specific acquisition programmes for perfusion imaging. Radiation dose rates and x-ray beam energy profiles have been altered in order to optimise the balance between image quality and radiation dose. In a small pilot study, ten patients have been imaged with a physiological assessment of blood flow (via intracoronary combined pressure and blood velocity measurements) and preliminary perfusion imaging protocol. Analysis software was written to produce quantitative measurements of myocardial blood flow from the image sequences.

### *Results*

Patient skin radiation dose rates have been reduced by 40% compared to standard angiographic protocols with almost no loss in image quality. Analysis of time-density curves did not correlate well with physiological measures of blood flow (e.g. gradient at half peak compared with Fraction Flow Reserve,  $r^2 = 0.11$ ), but ratio of pre- and post- intervention angiographic measures did correlate well with equivalent ratios of pressure measurements ( $r^2 = 0.97$ ). Angiographic measurements of blood mean transit time show initial promise, although there were too few cases for a full assessment.

### *Conclusions*

Further developments of the x-ray angiographic perfusion imaging protocols are required in order to provide a more complete assessment of blood flow in the myocardium.

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**List of non-presenting attendees:** Saif-El-Dean Abdel-Rahman, Abdullah Abualssayl, Sami Amudarra, Sumia Bageghni, Ester Collado Fernandez, Esther Cooke, Cheryl Craigs, Victoria Gatenby, Tao Jiang, Claire Keeble, Rhodri King, Christopher Lethaby, Ben Mercer, Nadeem Mughal, Victoria Richardson, Mark Tatterton, Andrew Woolston, Alia Al-Naji, Heather Leggett.

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