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School of Life and Medical Sciences Research Conference
Tuesday 4 April 2017
Lindop Building, College Lane Campus, University of Hertfordshire

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CONFERENCE PROGRAMME

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09.40 – 10.20	Plenary Lecture 2: Dr Angel Chater (University of Bedfordshire) *Behavioural Science in a Public Health Setting: Health, Wellbeing and Behaviour Change* Chair: Dr Mike Page, Reader in Psychology, Head of CRiPSS
10.20 – 10.40	Coffee Break
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ABSTRACTS OF ORAL COMMUNICATIONS

GUEST/PLENARY LECTURES

PLENARY LECTURE 1:

Sir Anthony Coates,
St George's, University of London

Will highly resistant bacteria destroy modern Medicine?

Chair: Dr David Griffiths, Principal Lecturer Chemistry

Abstract:

Antimicrobial Resistance (AMR) threatens the practice of modern Medicine. Surgery, cancer therapy and the treatment of bacterial infections depend on effective antibiotics. No new classes of antibiotics have reached the market for Gram-negative bacterial infections such as E. coli and Klebsiella for over 30 years. In intensive care units in India, Turkey and South-Eastern Europe, including Greece and Bulgaria, highly resistant Gram-negatives now affect about one in four infected patients. Only one antibiotic called colistin is broadly active against these resistant Gram-negatives. Now colistin resistance is widespread. Pan-resistant Gram-negative infections are still rare but are occurring already. The United Nations has recently passed a resolution which identifies AMR as an area of major public health concern.

Big pharma have moved out of the antibiotic discovery area, mainly due to the fashion for a low unit price for antibiotics. Small antibiotic Biotechs are beginning to emerge. Helperby Therapeutics is a small British Biotech, a spin out from St George's, University of London. Founded 15 years ago, it has an antibiotic renewable technology which boosts the effect of old antibiotics to kill highly resistant Gram-negative bacteria.

Helperby has numerous new classes of boosters called Antibiotic Resistance Breakers (ARBs). ARBs can rejuvenate up to five different classes of antibiotics. In addition, Helperby has shown that it is possible to reuse the same antibiotic again and again with different new classes of ARBs. If used one after the other, at say 30-year intervals, this could represent the first sustainable renewable antibiotic solution. It is, we believe, a unique solution and is aimed at the 19 or so non-penicillin classes. Helperby has seven programmes ranging from Phase 1 ready, Phase 2 ready, Phase 2 to market ready, including a systemic treatment for highly resistant Gram-negative bacterial infections.

PLENARY LECTURE 2:

Dr Angel Chater,
University of Bedfordshire

Behavioural Science in a Public Health Setting: Health, Wellbeing and Behaviour Change

Chair: Dr Mike Page, Reader in Psychology, Head of CRiPSS Research Centre

Abstract:

A common question that is salient to the public health agenda is: 'How can health promotion and treatment efforts be improved to enhance the health and wellbeing of the nation?' The major epidemics of chronic disease now facing us, such as obesity, diabetes, coronary heart disease and cancer, suggest we need to focus our efforts on embracing a strategic shift from treatment to prevention, while also enhancing national wellbeing.

It has long been discussed that poor mental health is significantly linked to illness and disease. On the contrary, a life that is happy and meaningful has been found to be linked to levels of positive mood, health enhancement and life satisfaction.

Applying health psychology in public health settings to understand behaviour, assess the need of those in crisis and maximise the scale of the impact of public health interventions at a national level, can all aid in the mission to improve population health and wellbeing. With this in mind, this talk will give examples of research and practice in the areas of weight management, the use of food banks, and interventions that aim to enhance wellbeing through sport and physical activity.

PLENARY LECTURE 3:

Dr Mandy Nevel,
The Royal Veterinary College

Livestock production – challenges and opportunities

Chair: Dr Yongju Huang, Reader in Plant Pathology

Abstract:

The global demand for animal protein is increasing. Not only is the human population growing but also there is an increasing demand by some countries as their economic situation improves. The growing demand will be met, at least in the short term, by species that are prolific and have high growth rates. Intensification and expansion of pig and poultry production systems is occurring in an attempt to meet this demand. At the same time, and particularly in developed countries, there is an increased demand for 'high welfare' or 'less intensively reared' produce. We have therefore seen a rise in the number of small holder/hobby units rearing smaller numbers of pigs and poultry on extensive systems. These smallholder units are a small but nevertheless important part of the UK system. Both systems bring their own benefits and issues. This paper will look at some of the issues in intensive production systems and outline where future research needs are likely to arise.

Intensive, and commonly, high density systems, rely on rapid throughput of large numbers of animals. Young, immunologically immature animals are more likely to suffer from outbreaks of disease. Treatment and control of these will often require antimicrobial (AM) intervention. Prudent use of AMs is perhaps the biggest issue for the farming sector. Both the scientific and media communities outline the 'potential' risk of AM resistance (AMR) transfer between animals and humans, but there is little evidence to support this. We need to look at if, and how, AMR is moved between species and at the same time identify alternatives to AM use.

The pig is a natural scavenger and in many parts of the world, pigs utilise waste material from households, converting it into edible protein. From a disease perspective, this has substantial risks. However, to maximise growth rates of commercial pigs, sophisticated nutrition is required to ensure efficiency. Such feeding must consider, and control, the consequences of excess nitrogen, and other elements, excreted in faeces and spread on agricultural land.

Animal feed production utilises large areas of land with subsequent pressure on the environment. Climate change and deforestation are often described as consequences of intensive agriculture. Improving efficiency of pig production further would negate some of these consequences. Control of disease outbreaks would improve efficiency as well as improving the welfare of the animals. Improved and new vaccines would assist with this aim. However, diseases emerge, and will continue to emerge ensuring the need for us as scientists.

PLENARY LECTURE 4:

Mr Nikhil Vasdev
Hertfordshire and Bedfordshire Urological Cancer Centre, Lister Hospital, Stevenage, UK

Robotics in Surgery

Chair: Dr Shori Thakur, Principal Lecturer in Pharmacology

Abstract:

Robotic surgery, computer-assisted surgery, and robotically-assisted surgery are terms for technological developments that use robotic systems to aid in surgical procedures. Robotically-assisted surgery was developed to overcome the limitations of pre-existing minimally-invasive surgical procedures and to enhance the capabilities of surgeons performing open surgery.

A review of the latest developments in Robotic Surgery will be presented, including two exciting new projects being conducted at the University of Hertfordshire on Cytokine Guided Robotic Surgery and Robotic Haptic Feedback Development.

PARALLEL SESSION I

Disease Mechanisms, Drug Discovery and Delivery (A154)

PARALLEL SESSION I INVITED LECTURE

Dr Brett Cochrane,
Science Director, Animal Free Research UK

Funding the development and application of human models for human disease

Chairs: Dr David Chau and Dr Stewart Kirton, Pharmaceutics and Medicinal Chemistry

Abstract:

Animal Free Research UK (formerly known as the Dr Hadwen Trust (DHT)) is a well-established charitable organisation that is fully focussed on developing and applying animal-replacement approaches to benefit human health. The DHT was originally founded in 1970, and since that time has funded more than 200 different research activities. Over the last 5 years alone Animal Free Research UK has invested over £4 million in the development, validation and application of animal-replacement technologies.

Animal Free Research UK recognises the importance of implementing animal-replacement approaches that feature the amalgamation of multiple scientific disciplines to meaningfully emulate human pathophysiology. This presentation will provide a brief overview of Animal Free Research UK and introduce the charity's various funding streams that may help support the development of human models of human disease.

STUDENT PARALLEL SESSION I

Chairs: Noelia Perez Diaz, Daniel Baker and Pushpendra Goswami

IN SILICO STUDIES TO IDENTIFY THE NEXT GENERATION OF NEW PYSCHOACTIVE SUBSTANCES (NPS)

Botha, M, Fergus, S., Stair, J.L. & Kirton, S.B.
Department of Pharmacy Pharmacology and Postgraduate Medicine, School of Life & Medical Sciences, University of Hertfordshire, UK

Introduction: The rapid emergence of New Psychoactive Substances (NPS) has seen an alarming number of fatalities and drug overdoses in a very short period of time. As such, these substances pose a sustained threat to public health[1], despite legislation introduced in April 2016 designed to make these previously legal psychoactive compounds illegal. Understanding how these compounds interact in the body, and what the next wave of NPS could be, is fundamental to help combat the threat to public health. Most NPS interact with monoamine transporter (MAT) proteins within the body. It is well established that there is a high degree of ligand promiscuity that occurs between the dopamine (DAT), norepinephrine (NET) and serotonin (SERT) transporters. The utilisation of *in silico* methods in rational drug design has become increasingly popular. The aim of this study was to use computationally-derived Quantitative Structure Activity Relationships (QSAR) models to investigate the protein-ligand interactions (PL-Is) of NPS in the monoamine transporter proteins to identify potential chemical features that elicit psychoactivity, and hence predict the next generation of chemical scaffolds that could appear.

Methods: Using Tanimoto coefficients and MACCS structural keys 31 NPS with known biological activity were divided into training and test sets. A range of different molecular descriptors were calculated for the 31 NPS ligands. The test and training set were used alongside the QuaSAR application in the MOE[2] software to build, QSAR models.

Results: QSAR models were built for the three MATs using isoform specific training sets (25 ligands) to build and test (6 ligands) the QSAR models. Models were constructed using the fewest possible number of molecular descriptors. Model quality was assessed by considering the r^2 value (which indicates the predictive ability of the model) the q^2 value (which accounts for how robust the model is) and how well the model accurately predicted the pKi values of the compounds in the test set. An r^2 of 0.68 and q^2 value of 0.61 was obtained for the DAT QSAR model and r^2 and q^2 value of 0.87 and 0.73 respectively for the SERT QSAR model. The r^2 and q^2 values for DAT and SERT indicate the models were robust and predictive. However, this was not the case for the QSAR model for NET (r^2 = 0.49, q^2 =0.30).

Conclusions: Robust and predictive QSAR models for two MATs (DAT and SERT) were generated. These provide testable hypotheses to investigate potential selectivity between the proteins based on the chemical properties of a ligand. The QSAR model for the third MAT (NET) was not predictive. This is likely as a result of limitations associated with the NET dataset.

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IDENTIFYING LEAD COMPOUNDS THAT PREVENT S100P AND RAGE BINDING AS A NOVEL THERAPY FOR PANCREATIC CANCER

Ogbeni, D.[1], Camara, R.[1], Chau, D.Y.S.[1], Patel, P.[1], Crnogorac-Jurcevic, T.[2], Kirton, S.B.[1], Mackenzie, L[1]., Rossiter, S[1].

1. Department of Pharmacy Pharmacology and Postgraduate Medicine, School of Life & Medical Sciences, University of Hertfordshire, UK

2. Barts Cancer Institute, Queen Mary University of London, UK

Background: Pancreatic ductal adenocarcinomas (PDAC), an aggressive and one of the most lethal human cancers, with a 5-year survival of less than 5% due remains a major challenge in oncology. High prevalence of a calcium-binding protein S100P has been shown to promote PDAC cancer progression through its interaction with the receptor for advanced glycation end products (RAGE). As such, S100P has emerged as a promising biological target for novel anticancer drug design. This project aims to identify lead compounds that attach to and prevent S100P from binding to and activating RAGE.

Methods: An in-house developed enzyme linked immunosorbent assay (ELISA) was used to confirm the binding of S100P with RAGE and assess the efficacy of 93 synthesised compounds. All 93 compounds with concentrations ranging from 1μM-1nM, were screened in triplicate. Selected candidates were tested for their ability to alter cell metabolic activity using MTS (metabolic activity) and LDH release (cell toxicity) assays. In addition, Transwell cell migration and invasion studies were performed using pancreatic cancer cells with (BxPC-3) and without (Panc-1) endogenous S100P expression to assess the migratory and invasive properties of these cells after treatment with the lead compounds.

Results: MTS and LDH release assays revealed that the compounds did not exhibit general cytotoxicity. 18 compounds demonstrated positive S100P/RAGE inhibitory characteristics. Cell migration and invasion studies revealed that, a 48-hour treatment with 13 of the 18 lead compounds at 10μM also demonstrated a significant reduction in cell migration and invasion of BxPC-3 cells but not in Panc-1 cells.

Conclusions: Results from this study confirm that the measurement of S100P-RAGE binding by ELISA can be used as a screen to identify compounds that have functional effects in S100P-expressing pancreatic cancer cells and enable further development of a potential therapy for pancreatic cancer.

DESIGN AND VALIDATION OF A LOWER AIRWAYS *IN VITRO* MODEL INCORPORATING INNATE IMMUNE CELLS FOR TOXICITY SCREENING

Martin, **A.**

Supervisors: Dr Darragh Murnane, Dr David Chau, Prof. Marc Brown & Dr Victoria Hutter.

Department of Pharmacy, Pharmacology & Post-graduate Medicine, School of Life & Medical Sciences, University of Hertfordshire, UK

Introduction: Lung disease such as asthma and chronic obstructive pulmonary disease are increasing the global heath burden affecting hundreds of millions of people worldwide. The development of novel inhaled therapies is being hindered by the lack of understanding of alveolar macrophages (AM) to inhaled particulate medicines. In animal models, there is increasing evidence that inhaled medicines are taken up by AM resident within the lung resulting in poorly understood perturbations. This means that candidate drugs may fail early in preclinical screening despite not knowing whether these effects are adverse or not in humans. The aim of this work is to identify an appropriate *in vitro* AM culture model for the generation of a human co-culture model to measure toxicity of inhaled compounds.

Methods: The U937 human monocyte cells lines were purchased from the LCG Standards and cultured in supplemented RPMI medium. For differentiation into an AM phenotype, cells were seeded onto 96-well plates at a density of $5x10^5$ cells/mL in complete culture medium supplemented with phorbol myristate acetate (PMA) (5-100 nM) with a range of incubation and resting times of 24-96 h and 24 h respectively. Differentiation was validated using CD marker array analysis and surface marker expressions by flow cytometry. Antibodies used were fluorescently conjugated to mouse anti-human CD14, CD11a, CD11b, CD206 and CD36 with isotype controls obtained from BD Biosciences as previously described. May-Grünwald Giemsa stains were employed for morphological assessment of cells.

Results: Protein microarray results showed that PMA treated U937 cells were rich in surface markers specific to macrophages and AM. Cell surface marker expressions for CD11a, CD11b and CD36 were significantly (p<0.05) more abundant in the PMA differentiated U937 cells in comparison with undifferentiated cells at a high and low passage number. A significant decrease (p<0.05) in phagocytic activity was observed for all treatments with increasing time in culture. Prolonged viability and cell growth arrest of 100 nM PMA treated U937 cells was evident for at least 18 days after 24 h and 96 h differentiation methods unlike PMA naïve and 5 nM PMA treated cells. Morphological differences including increased pseudopodia were observed in cells treated with 100 nM PMA in comparison with exposure to 5 nM concentrations.

Conclusions: The concentration of PMA and incubation time used for the differentiation of the U937 cell line is an important factor in generating AM-like cells. We have shown that variations in the differentiation protocol for U937 result in cells with altered levels of differentiation markers, proliferation characteristics and morphology. Validation and characterisation of this cell line will allow for the generation of an epithelial-macrophage co-culture model that can be developed to understand human AM responses to inhaled pharmaceuticals and the link between cellular cross talk in the lower airways.

SYNTHESIS AND EVALUATION OF NOVEL ANTIFUNGAL AGENTS TARGETED TO THE PLASMA MEMBRANE H^+-ATPase ENZYME

Patel D. V.

Supervisors: Dr. J. Paul. Bassin & Dr. David. G. Griffiths.

Department of Pharmacy, Pharmacology & Post-graduate Medicine, School of Life & Medical Sciences, University of Hertfordshire, UK.

Introduction: Fungal infections now contribute more significantly to microbe-associated morbidity and mortality due to limited number of available antifungals [1]. Over the last two decades many pathogenic fungi have developed various modes of resistance [2]. Since 2002, only echinocandins have been introduced as a new class of antifungal. Many presently used clinical antifungals (e.g. polyenes) have adverse side effects along with limited modes of drug delivery. There is thus an increased need to identify novel drug targets within the mycota. The plasma membrane (PM) H^+-ATPase is an essential enzyme for the growth of fungi where it maintains an electrochemical proton gradient across the fungal plasma membrane [3]. The PM H^+-ATPase has been shown to be inhibited by sulphinimides (e.g. omeprazole), epsilon and *α,β*-unsaturated carbonyls such as curcumin [4,5]. These encouraging results prompted us to synthesize a dieneone library similar to curcumin and to investigate their antifungal activities.

Method: Using the Claisen-Schmidt condensation a dieneone library was synthesized between N-methylpiperidin-4-one or cycloalkanones with various substituted aldehydes. These compounds were characterized using LCMS, FTIR, ^1H and ^{13}C NMR and screened for their antifungal activity against *Saccromyces cerevisiae* and *Candida albicans* using a macro broth susceptibility assay. Test and control samples were incubated at 32°C and 100 rpm for 24 hours. Optical density was measured at 600nm. EC_{50}, slope values, curve symmetry and AUC were obtained from the dose-response curves fitted to four and five parameter logistic equations. These compounds are presently being screened against other species of *Candida*. Also H^+ extrusion assays are being performed to narrow down the enzyme targets.

Results: The pyridylidene and substituted benzylidene derivatives of N-methylpiperidin-4-one, cyclopentanone and cyclohexanone exhibited a wide range of inhibitions against *S. cerevisiae* (values for EC_{50} ranged from 0.6 to 23 µM). However, thienylidene derivatives of N-methylpiperidin-4-one, cyclopentanone and cyclohexanone were not effective inhibitors of *S. cerevisiae* (EC_{50}= 2233 µM, 619 µM and no inhibition, respectively). Inhibition of *C. albicans* growth ranged from no inhibition (thienylidene derivatives) to 50 µM (pyridylidene derivatives of cyclopentanone and cyclohexanone).

Conclusion: The compound 3,5-bis((E)-(4-trifluoromethylbenzylidene)-1-methylpiperidin-4-one was the most potent inhibitor against *S. cerevisiae* (EC_{50}=0.62±0.26 µM), whereas 2,6-bis-[(E)-(4-pyridyl)methylidene]cyclohexanone showed the highest activity against *C. albicans* (EC_{50}=50±3.50 µM). These results offer an encouraging framework that could lead to the discovery of novel antifungal agent which inhibits the plasma membrane H^+-ATPase.

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THE IMPACT OF NOVEL PSYCHOACTIVE SUBSTANCE MISUSE ON ACUTE MENTAL HEALTH SERVICES

Shafi, A. [1,2], Gallagher, P.[1], Stewart, N.[1], Corazza, O.[2]

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[2]Psychopharmacology, Drug Misuse & Novel Psychoactive Substance Research Unit, School of Life & Medical Sciences, University of Hertfordshire, UK

Introduction: Novel Psychoactive Substance (NPS) misuse is emerging as a serious threat to mental health services, as observed in the increasing number of anecdotal reports currently being generated, yet evidence on their impact is limited.[1] They are synthetic substances that have been developed to produce altered states of consciousness and perception, and in essence to mimic existing recreational drugs such as cannabis, cocaine and ecstasy.[2] Those with a diagnosis of mental illness are more likely to engage in NPS misuse than those without mental illness, but the short and long term effects of NPS are largely unknown.[3] This research study was developed to understand the impact of NPS misuse on acute mental health services and compare admission characteristics with non-NPS substance misuse and non-substance misuse, and identify key differences that will affect treatment and management.

Methods: Assessment of all patients admitted to a 120 bed inner London mental health facility from March 2016 to date. Patients have been screened by a multi-disciplinary team of healthcare professionals, using a unique and innovative 10 question assessment tool specifically created for the study, and one designed to collect new data on NPS misuse. The tool has adopted the mnemonic “PASS SAFELY” to ease utilisation by healthcare professionals and to serve as a reminder that these service users should not suffer from harm during their admission process.

Results: NPS use has been identified in 13.1 % of admissions. In comparison to non-NPS users, NPS users were statistically more likely to be involved with violence pre-admission ($p<0.001$, 10.51 (95% CI 5.03 to 21.92) and violence during admission ($p<0.001$, 15.93 (95% CI 7.52 to 33.77). There is also a strong correlation with increased length of stay in hospital and readmission rates for NPS misusers when compared to non-NPS misusers and non-substance misusers. An irreversible impact on the cognition of new NPS misusers has been observed to be a limited but major finding.

Discussion: The results indicate an immediate need for inpatient and community mental health services to ensure targeted assessment and risk management strategies for NPS misusers to reduce the risk of violence and harm towards others. The finding of irreversible impact on the cognition of young new NPS misusers presents major challenges not just to mental health services but to public health services in general, as currently there is no service provision for young adults presenting with a clinical picture similar to that of moderate to severe dementia.

References:

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PARALLEL SESSION II

Health and Wellbeing (Room A166)

PARALLEL SESSION II INVITED LECTURE

Dr Lettie Bishop,
Loughborough University

Exercise and reducing systemic inflammation in kidney disease – if only exercise was a pill…

Chair: Dr Lindsay Bottoms, Senior Lecturer in Human Physiology

Abstract:

Chronic Kidney Disease (CKD) is an irreversible, gradual decline in kidney function which affects around ~8% of adults (~1.8 million) in the UK. Treatment and care of patients with CKD accounts for around 2% of the NHS budget (~£1.5 billion/year) and as such is increasingly being recognised as a major public health problem.

Heart disease and infection are major complications and the leading causes of death in these patients. It is now well established that immune system dysfunction is involved in both of these pathological processes. Specifically, impaired immune function predisposes to infection, while persistent immune activation leads to a state of chronic systemic inflammation that can damage the insides of blood vessels and increase heart disease risk.

Physical exercise may confer benefits by exerting anti-inflammatory effects and enhancing immunity, but such effects have been largely unexplored in kidney disease and the development of formal rehabilitation programmes for these patients lag behind those of other chronic conditions, such as heart disease, lung disease and diabetes. In this talk data will be presented from our studies examining the impact of exercise on markers of systemic inflammation and immune function in patients at all stages of kidney disease; non-dialysis, dialysis and most recently, renal transplant recipients.

STUDENT PARALLEL SESSION II

Chairs: Camilla Holland, Nick Shipp and Lynsey Northeast

ADHERENCE BEHAVIOUR IN HAEMODIALYSIS PATIENTS' PRE AND POST RECEIPT OF A KIDNEY TRANSPLANT: A RETROSPECTIVE STUDY

Hucker, A[1]; Sharma, S[1]; Farrington, K[2]; Lawrence, C[2]

[1] Department of Psychology & Sport Sciences, School of Life & Medical Sciences, University of Hertfordshire, UK

[2] Renal Unit, Lister Hospital, Stevenage, Hertfordshire, UK

Introduction: Adherence to immunosuppressants is essential for renal transplant recipients. In this retrospective study based at the Lister Hospital in Hertfordshire, we were interested in exploring whether adherence behaviour on haemodialysis is indicative of patterns of adherent behaviour post-transplantation.

Methods: Clinical data was retrieved for 86 patients (65.1% male). Patients were only included in this study if they received a transplant after 2006 and therefore electronic notes from clinic visits were available. Data was retrieved from the six-month period prior to transplantation, and one year post-transplantation, following return transfer to the Lister Hospital transplant clinic from the transplanting hospital. Patients were only included if they were transferred back within the first year following transplantation. Pre-transplant data elements included demographic and clinical data, including age at first dialysis session, average variance from dialysis prescription and number of missed dialysis sessions over the 6 months prior to transplantation. Post-transplant data included donor type, average tacrolimus level and number of missed transplant clinic appointments over one year post-transplantation, following return transfer from the transplanting hospital.

Results: Pre-transplant data was coded for 86 patients. The mean age at the first dialysis session for the overall sample was 44.02 years (*SD* = 13.50). Patients were identified as non-adherent if they on average shortened their dialysis prescription by >10 minutes over the six-months prior to transplantation. Within the sample, 55 (64%) were identified as adherent and 31 (36%) as non-adherent. The mean variance from dialysis prescription over six months for the non-adherent group was -26.46 minutes (SD= 24.39), and 16.2% had missed two or more haemodialysis sessions in the six-months prior to transplantation.

Post-transplant, the mean tacrolimus level for the overall sample was 8.40 ng/mL (*SD* = 1.83). Around 22.1% of patients had missed one or more transplant clinic appointments within the first year following return transfer from their transplanting hospital, and 31.4% of patients experienced delayed graft function. Of the 86 patients, 14 (16.3%) had tacrolimus levels outside of the range expected within the first two years of 5-10 ng/mL, of which 42.9% had missed one or more post-transplant clinic appointments within the first year following return transfer. Of these 14 patients, almost all averaged less time than their dialysis prescriptions over the six months prior to transplantation, with a mean variance of -10.53 minutes (*SD* = 15.40).

Conclusions: Non-adherence to immunosuppressive medication is a major risk factor for poor clinical outcomes post-transplantation and has been identified as a common issue in this patient population. Initial descriptive, retrospective findings suggest that a notable proportion of patients have (a) missed dialysis

sessions in the six months leading up to transplantation (b) go on to miss post-transplant clinic appointments and (c) have tacrolimus levels outside of the suggested range. There is emerging evidence to suggest that some pre-transplant adherence behaviours may be related to post-transplant adherence also. It is important, therefore, to identify when and how to intervene to support patients in gaining optimal outcomes from receipt of a solid organ transplant.

THE EFFECTS OF MONTMORENCY TART CHERRY JUICE SUPPLEMENTATION ON FAT OXIDATION DURING FATMAX EXERCISE AND CARDIO-METABOLIC MARKERS AT REST

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Introduction: Montmorency tart cherries (*Prunus cerasus*) are rich in anthocyanins (Kirakosyan et al., 2009), compounds capable of augmenting fat oxidation and regulating metabolic dysfunction (He and Giusti, 2010). Aerobic exercise at individual FATMAX (conveyed as percentage of maximal oxygen uptake (%VO2max)) has been shown to alleviate symptoms associated with Metabolic Syndrome (MetS), partly through enhancing fat oxidation rates (Brun, Romain and Mercier, 2011). As this was the first study to incorporate exercise and Montmorency tart cherry juice (MTCJ) supplementation in tandem, healthy participants were recruited to observe cardio-metabolic responses and assess for any adverse effects. The present study examined whether MTCJ supplementation could augment fat oxidation rates during FATMAX exercise and mitigate symptoms of MetS in healthy participants.

Methods: Eleven healthy, recreationally active, participants (18-45 years) consumed MTCJ (30mL concentrate with 100mL water) or placebo (PLA) twice daily, in a random, counterbalanced order for 20 days. Participants cycled at their individual FATMAX (determined using an incremental protocol adapted from Achten, Gleeson and Jeukendrup, (2002)) for 1 hour pre-, mid- (10 days) and post-supplementation during which substrate oxidation rates were measured. Waist circumference, body composition and resting metabolic rate were measured pre-post exercise. Blood pressure, serum triglycerides, cholesterol, HDL, total antioxidant status (TAS), glucose and lactate were measured immediately before and after the 1 hour exercise. LDL was later calculated using the formula by Ahmadi et al. (2008).

Results: No significant differences ($P>0.05$) between conditions or interactions were observed for any blood-based biomarkers, blood pressure, waist circumference, body composition or fat (Figure 1) and carbohydrate oxidation rates during exercise or rest.
Pre-exercise TAS ($P=0.036$) and HDL ($P=0.001$) values were significantly reduced from mid- to post-supplementation with MTCJ but not PLA.

Discussion: Supplementation of MTCJ for 20 days did not augment fat oxidation rates at rest or during FATMAX exercise in healthy, recreationally active individuals. These results were likely confounded by the high carbohydrate content of MTCJ. Beyond 10 days' supplementation, MTCJ could not maintain elevated TAS and HDL levels. In line with previous research (Lynn et al., 2013), blood-based and functional cardio-metabolic markers associated with MetS were not significantly improved, likely due to the healthy baseline values and high inter-individual variability presented by the participants. Therefore, it is unnecessary to supplement MTCJ in this participant cohort to improve MetS symptoms.

Acknowledgements: Funding was provided by Santander UK and University of Hertfordshire Diamond Fund. Supplements were provided by The Cherry Research Committee.

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KEEPING A DIARY OF INTRUSIVE MEMORIES: THERAPEUTIC EFFECTS IN A NON-CLINICAL SAMPLE

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Introduction: Intrusive memories (IM) can be defined as "spontaneous involuntary memories of a (mostly) negative event that repeatedly intrude upon consciousness, often against one's will: they are hard to control and may disrupt one's ongoing activities" (Kvavilashvili, 2014, p.101). Most research on IM has been conducted using retrospective reports obtained via interview or questionnaire. A structured diary has been shown to be a successful method for studying (non-intrusive) involuntary autobiographical memoires (IAM) but has been used only a limited number of times to study IM (Kleim, Graham, Bryant, & Ehlers, 2013; Williams & Moulds, 2007). Furthermore, evidence from expressive writing (Pennebaker, 1997) and IAM (Boals, Hathaway, & Rubin, 2011) research suggests that recording IM in a structured diary may produce therapeutic benefits. Preliminary exploration of this effect has produced evidence suggesting the same (Kvavilashvili & Brewin, 2013). The present study aims to further explore this effect, and distinguish it from that of other types of writing interventions, namely the administration of one-off memory questionnaires.

Methods: Participants (n=56) were recruited based on the report that they experience at least one IM per day, and were randomly allocated to one of three conditions. In Condition 1 participants completed a preliminary questionnaire about their IM at an initial meeting, and then recorded their IM in a diary for 2 weeks. Condition 2 participants did not complete the IM screening questionnaire, but then recorded their IM in a diary for 2 weeks. The third (control) condition participants completed the preliminary IM questionnaire, but then recorded their involuntary prospective memories in a dairy for 2 weeks. Before and after the 2-weeks of diary keeping, participants completed the State-Trait Anxiety Inventory (STAI), Beck Depression Inventory (BDI), and the PTSD Check List – Civilian Version (PCL-C).

Results: Only the PCL-C scores reduced significantly between the first and second completion, but group differences were not as anticipated, with the control (prospective memory diary) condition registering the greatest drop in score.

Discussion: It may be the case that, to produce a therapeutic benefit, there is an optimal period of time for engaging with the contents of an intrusive memory, and that 2 weeks of diary keeping exceed this.

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WRIST-WORN ACCELEROMETER MEASURES OF MOVEMENT BY PEOPLE WITH PARKINSON'S DURING AND FOLLOWING DANCE CLASSES AT THE UNIVERSITY OF HERTFORDSHIRE

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Introduction: People with Parkinson's have anecdotally reported that the physical and psychological benefits of attending a dance class are maintained for the hours immediately following the class; however these may wear off over the subsequent days. The use of a wrist-worn accelerometer can provide an objective, yet non-invasive, way of quantifying the amount of movement made by people during and after such activities (Eslinger, Rowlands, Hurst, Catt, Murray & Eston, 2011). To date, research has focused on using the devices to classify specific movements, such as tremor, over a short period of time. The purpose of the current study is twofold: firstly, to measure the amount of movement made by people with Parkinson's compared with age-matched controls and younger individuals during a dance class; secondly, to measure the amount of movement by people with Parkinson's over the week following a dance class compared with a week when they do not attend a dance class.

Methods: People with Parkinson's and age-matched controls who regularly attend a dance class at the University of Hertfordshire were asked to wear an accelerometer on their wrist as they took part in a dance class and then on a separate occasion for seven days following the dance class. They were not asked to do anything specific other than continue their daily routine as normal and to keep a brief diary of their activity over the seven days. Participants then wore the accelerometer during a week when they did not attend a dance class to allow a comparison between the amount of movement made on a dance and non-dance week. In addition, data collection is currently underway over a week for people with Parkinson's who do not attend dance classes.

Results: Feedback from the participants has been positive about wearing the accelerometers, indicating the feasibility of using this device to measure activity over a sustained period of time. Accelerometer measures revealed that during the dance class people with Parkinson's (N=12) moved to the same extent as age-matched controls (N=12), but less than younger individuals (N=12, $P<0.05$). Preliminary exploration of the seven day data suggests that people with Parkinson's move less over the course of the week following attendance at a dance class compared to age-matched controls. Further analyses when data collection is complete will investigate whether this group difference is significant and whether people with Parkinson's are more or less active during the week following a dance class compared with a week when they did not attend a dance class.

Discussion and Conclusions: Wrist-worn accelerometers are a feasible way of tracking the level and pattern of activity made by people with and without Parkinson's over the week following a dance class. Initial results from the seven day study mirror previous research which found that self-reported activity levels by people with Parkinson's were less compared to age-matched controls (van Nimwegen et al.,

2011). Therefore, the devices may be useful as a means of quantifying over the longer term the reduced activity levels that are a characteristic motor feature of Parkinson's.

Acknowledgements: The researcher was funded by a Department of Psychology and Sports Sciences PhD bursary.

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SEE IT, FEEL IT, OWN IT: HOW GALVANIC VESTIBULAR STIMULATION ENHANCES BODY OWNERSHIP DURING THE RUBBER HAND ILLUSION

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Introduction: Body ownership refers to the sense that one's body belongs to oneself. This facet of bodily self-consciousness relies on the integration of sensory signals from several different sources, including traditionally exteroceptive information (e.g. vision) and interoceptive information (e.g. proprioception and affective touch). The vestibular system plays a key role in managing the balance between various sensory systems and their contribution to body ownership. However, it is still unclear how exactly these different sources of information contribute to body ownership. The aim of the current study was to examine how the vestibular system affects the relative influence of these different sensory modalities to body ownership.

Methods: We used galvanic vestibular stimulation (GVS; Ferrè, Berlot, & Haggard, 2015; Lopez, Lenggenhager, & Blanke, 2010) to stimulate the vestibular system of healthy participants during an experimentally induced body ownership illusion (i.e. the rubber hand illusion; RHI; Botvinick & Cohen, 1998). Participants took part in a RHI procedure, during which they observed a realistic rubber hand being stroked in or out of synchrony with their own unseen hand. This synchronous (but not asynchronous) stroking typically increases ownership of the rubber hand (as indexed via questionnaires and the perceived drift in location of the real hand towards the rubber hand; i.e. proprioceptive drift). We compared rubber hand ownership in 26 healthy participants during conditions of: (1) vestibular stimulation resulting in activation of the right- vs. left-hemisphere of the brain vs. sham stimulation, (2) synchronous vs. asynchronous stroking of the real and rubber hand, and (3) affective (slow) vs. neutral (fast) velocity stroking. This resulted in a 3 (GVS stimulation) x 2 (stroking synchrony) x 2 (stroking velocity) within-subjects design. In addition, to examine the effect of vestibular stimulation during vision alone (i.e. without tactile stimulation) on body ownership (referred to as 'visual capture') we performed the same three types of stimulation while participants simply looked at the rubber hand.

Results: We found that right-hemisphere stimulation (as compared to left hemisphere or sham stimulation) significantly increased visual capture (i.e. there was a significant increase in proprioceptive drift) during the vision only conditions. In the touch conditions, right-hemisphere stimulation during synchronous stroking also increased proprioceptive drift compared to the stimulation of the same areas in left hemisphere. Finally, right-hemisphere stimulation resulted in affective (i.e. slow, gentle) touch leading to a significant increase in proprioceptive drift compared to neutral (i.e. fast) touch during synchronous stroking conditions.

Conclusions: Our study revealed that right-hemisphere vestibular stimulation influences the balance of the different sensory modalities contributing to the sense of body ownership. Specifically, right-hemisphere vestibular activation enhances visual information during the rubber hand illusion, thereby reducing the

influence of proprioceptive signals. In addition, using slow, affective touch during right-hemisphere activation further enhanced the dominance of visual information over proprioceptive signals. Our findings highlight the importance of the vestibular system in managing the balance of sensory information that contribute to body ownership, and suggest an important role of affective information (i.e. slow gentle touch) in the way we perceive our body.

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PARALLEL SESSION III

Biological Mechanisms in Pathogens and their Hosts (Room A161)

PARALLEL SESSION III INVITED LECTURE

Dr Cristina Barrero-Sicilia,

Rothamsted Research, Harpenden

Germination in domesticated and wild Triticeae seeds: comparison between *Brachypodium distachyon* and *Hordeum vulgare*

Chair: Dr Georgia Mitrousia, Post-Doctoral Research Fellow

Dedicated to Prof. Pilar Carbonero upon retirement

Centro de Biotecnología y Genómica de Plantas (UPM-INIA), and ETSI Agrónomos, Campus Montegancedo, Universidad Politécnica de Madrid, Pozuelo de Alarcón, 28223-Madrid, Spain.
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Abstract:

During the maturation phase of development, the cultivated Triticeae seeds (barley, wheat, etc.) accumulate abundant reserves (mainly proteins and starch) in the endosperm that will be hydrolised upon germination. Although phylogenetically related, *Brachypodium distachyon* seeds are characterized by low levels of starch and high levels of β-1,3-1,4-glucans in the endosperm cells, and thick mannan-rich cell walls in the coleorhiza and root.

The germination process, both in barley and Brachypodium, can be separated into two phases: 1) germination *sensu stricto*, when the coleorhiza is the first structure that protrudes after the pericarp and testa rupture (coleorhiza emergence), followed by the root emergence when the coleorhiza ruptures, and 2) post-germination reserve mobilization, when proteins and other reserves are hydrolised. Immunolocalization of mannans indicate their presence in the root embryo and in the coleorhiza in the early stages of germination, decreasing thereafter; at the same time, a pick of endo-β-mannanase activity, and the expression of mannanase encoding genes are observed. If the coleorhiza in monocots and the micropylar endosperm in dicots have similar functions and the importance *of BdMAN* genes in both type of seeds during germination *sensu-stricto* is discussed.

In post-germinating reserve mobilization, water imbibed seeds synthesize GA in the embryo which diffuses to the aleurone layer where it triggers the expression of hydrolase genes, such as *BdCathB*, encoding a CathepsinB-like protease, that is regulated by the Transcription Factors (TF) BdDOF24 and BdGAMYB.

The structure and function of these Brachypodium TFs and their orthologs in barley HvDOF24 (BPBF) and HvGAMYB are compared.

Keywords: Germination *sensu stricto*, Reserve mobilization, Endo-β-Mannanase genes *(MAN),* CathepsinB genes (*BdCathB*), Transcription Factors (TF).

STUDENT PARALLEL SESSION III

Chairs: Coretta Kloppel, Chinthani Karandeni Dewage and Unnati Shah

ANTIMICROBIAL PROPERTIES OF SOME AFRICAN (GHANAIAN) PLANTS USED IN TRADITIONAL/FOLK MEDICINE FOR THE TOPICAL TREATMENT OF INFECTIONS

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Background: Antibiotic resistance is a current worldwide problem with health, political and social consequences. The use of plant extracts in the treatment of various infections has been part of the culture of most rural communities in Ghana. In modern times, claims have been made of cures from herbal treatment where modern medicines have failed. With the current antibiotic resistance worldwide research into the possibility of new generation of antibiotics of plant origin is growing.

Method: Twelve dry plant samples popularly used in traditional Ghanaian medicine were crushed and extracted with water, ethanol and chloroform. Each crude extract was tested for its antimicrobial activity on *Staphylococcus aureus B3*, *Streptococcus pyogenes*, NCTC 8193, *Klebsiella aerogenes B14 NCTC8172*, *Pseudomonas aeruginosa B287 NCTC 10662*, and *E coli NCTC 9001.* disc diffusion method using extract concentration of 8.0mg/ml was used with Streptomycin 25µg standard disc as positive control. The 96 micro-litre well plate was used to determine the Minimum inhibitory concentrations and the minimum bactericidal concentrations with Streptomycin as the positive control. The active compounds in the extracts were isolated by thin layer chromatography and their activities against the test organisms compared with that of 25µg/ml. The chemical nature of the active ingredient were analysed by LC-MS.

Results and Discussion: Crude extracts from some of the plants, for example, *Alchornea cordifolia* showed activity against all the bacteria species with zones of inhibition ranging between 10mm and 20mm. *Morinda morindoides* and *Solanium eriantum* both showed activity against the *S aureus* spp, *Pseudomonas* and *E coli* spp with inhibition zones between 12- 15mm. MIC's of 0.125 -0.25mg/ml and MBC's of 0.25-0.5mg were recorded. Elutes of TLC bands of the extracts showed more pronounce activity giving inhibition zones of 15-20mm. These African medicinal plants contain some antimicrobial compound which can be possible lead to new antibiotic discovery. LC-MS ion chromatogram and mass spectra data of the active compound are being analysed.

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EVALUATION OF NON-NATIVE EUROPEAN CATFISH (*SILURUS GLANIS*) IN THE AQUACULTURE RISK ASSESSMENT SCHEME (ENSARS) IN THE UK

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Introduction: Risk assessment modules under the European non-native Species in Aquaculture Risk Assessment Scheme (ENSARS) were used to assess the risk of European catfish *S. glanis* in the UK to further understand their non-native species impacts. In recent years, introductions of *S. glanis* have increased due to angling pressures with increasing concern about their dispersal and colonisation into riverine ecosystems (Copp et al., 2009).

Methods: The assessment included the use of several ENSAR modules (Infectious Agent, Facility, Pathway and Socio-economic) to review the risk status of *S. glanis* impacts in the UK.

Results: Overall, module mean scores and confidence levels were variable for *S. glanis.* For the Facility module, they were categorized as medium risk 2.3(1.9), but moderately high for the Pathway module 2.5 (2.3) and ranked as moderately low 1.5 (1.8) for the Infectious Agent despite the presence of novel ancyrocephalid monogenean parasite *Thaparocleidus vistulensis* being detected on specimens. Similarly their socio-economic risks were ranked as low risk 1.5 (2.1) with eradication costs being fairly low with riverine deterioration caused by *S. glanis* unlikely.

Conclusion: Variability in mean scores and confidence levels in assessing risk status of *S. glanis* indicate that these study outcomes were preliminary evaluations and further risk assessments should be considered. In conclusion, the Facility, Pathway and Infectious Agent Modules highlighted the need for greater control measures by fishery managers in the importation and transfer of non-native *S. glanis* to lakes to avoid accidental introductions and spread of disease. Their low socio-economic risk is also variable as in riverine habitats *S. glanis* populations are not yet considered established but by contrast, there are reports of their colonisation in lakes throughout the UK. Further research is still required to address the lack of detailed information of species survivorship, dispersal and their non-native impacts in aquatic habitats in the UK.

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A HYDROGEOLOGICAL EXPLANATION FOR FORMATION OF SHALLOW DEPTH AQUIFERS IN THE FOOTHILLS OF THE WESTERN GHATS IN INDIA

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Introduction: Aquifers found at shallow depths in laterite terrain are one of the main drinking water resources in Asia and Africa. In many occasions these shallow depth aquifers have been vaguely defined as the water table of the area which fails to fully characterise their geohydrological properties. In Karnataka and Kerala these aquifers enable the existence of traditional water harvesting 'horizontal tunnel' system known as *Suranga*, and dugwells that are mainly found at moderate altitudes in hilly terrain where laterites are present. Information about the aquifer properties of laterite is sparse, and due to an absence of a clear understanding of water movement in laterite profiles it makes management of traditional water resources found in a laterite terrain difficult and vulnerable. The importance of *Suranga*, and shallow dugwells as cheap and reliable sources of drinking water to a large subsistence level population provides a clear rationale for better explanation and understanding of the hydrogeology of these shallow aquifers found in laterite terrains in the villages of the foothills of the Western Ghats in
India.

Methods: This study was a part of a larger study of a traditional water management system known as *Suranga* found in lateritic terrain in the foothills of the Western Ghats in India. Discharge measurement from traditional water management structures, which were found to be fed by these shallow aquifers were recorded for a year and compared with the local precipitation data that enabled an understanding of the relationship between rainfall and water availability in shallow aquifers. Water samples from *Suranga*, dug wells, ponds, natural caves, and bore wells were radiocarbon dated to analyse the age of various waters and to examine the water movement in subsurface substrates. Design principles of *Suranga*, shallow dug well, and lithology and rock profiles observation were done that were compared with the previous studies on hydrogeology of laterite in other parts of the world. It provided an understanding of the water movement inside laterite profiles.

Results: It was found that the water discharged from *Suranga* and other traditional water structures varied in accordance with the rainfall, and water availability fluctuates with the annual precipitation. However, water from deep wells and bore wells remain independent of seasonal weather change. The deepest water sample from 34 metres below the sea level of a bore well was found to be the oldest with age of 8440 ± 21.9 BP, and samples from a natural cave systems and a shallow dug well were found to be from Post 1950 CE, while water from *Suranga* was found to be 1830 ± 30 BP old. Rock and lithological analysis supported the idea of formation of several layers of varying subsurface permeability as a result of the chemical weathering of granite and gneiss rock profiles in the case study area.

Conclusions: *Suranga* and dug wells generally draw water from subsurface perched aquifers that are generally formed above the low permeable clay layers or hard duripan formed by long term weathering processes. These perched aquifers are recharged from precipitation, relatively quicker than deep water

tables, and are not directly connected to ground waters or deep water aquifers. This paradoxically make these sources more vulnerable to climate change, but less vulnerable to over-extraction.

MICROBIAL ECOLOGY OF A *PASTEURIA* SPECIES IN SCOTTISH SOILS

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Introduction: *Pasteuria* spp. spore forming bacteria which act as natural antagonists to many of the most damaging plant parasitic nematodes (PPNs). Highly specific nematode suppression may be observed in soils containing a sufficiently high density of *Pasteuria* spp. spores. This suppression is enacted by the bacteria *via* inhibition of root invasion and sterilisation of the nematode host. Molecular methods for the detection of *Pasteuria* spp. from environmental DNA (eDNA) have been described; however these methods are limited in both scale and depth.

Methods: We report a method of *Pasteuria* spp. community profiling based on second generation sequencing of primer bar-coded, species specific, PCR products. To test the utility of this method we have investigated *Pasteuria* spp. population structure in Scottish soils using eDNA from two sources: soil extracted DNA from the second National Soil Inventory of Scotland (NSIS2); and nematode extracted DNA collected from farms in the East Scotland Farm Network (ESFN). The NSIS2 data set is inclusive of comprehensive soil metadata which may be used to infer relationships between *Pasteuria* spp. environmental conditions. *Pasteuria* spp. community profiling in the ESFN data set was combined with similar nematode community profiling to attempt to establish whether host parasite relationships could be inferred from the parallel comparison of these data.

Results: Our results indicate that *Pasteuria* spp. populations in Scottish soils are broadly dominated by two operational taxonomic units (OTUs). The first of these OTUs clustering with high identity to *Pasteuria hartismeri*, a species first described parasitizing *Meloidogyne ardenensis,* a nematode parasite of woody and perennial plants in northern Europe. The second major OTU group clusters with a *Pasteuria*-like sequence which was first recovered from a farm near Edinburgh which was found to contain nematodes encumbered by spores of *Pasteuria* spp. for which a clear host range has not yet been identified. No clear non-specific amplification was observed with all sequences of appropriate length aligning with high identity (>95%) to one or more described species of *Pasteuria*. The number of reads recovered was scalable to the number of target gene copies included as template in controls, with clear and consistent detection of at least 1000 copies, and as few as ten.

Discussion and conclusions: Further statistical analyses are as yet required to establish if any of the abiotic factors measured may have any observable impact on the presence, or relative abundance, of *Pasteuria* species. When *Pasteuria* community profiles are combined with those of nematodes it may be possible to infer possible host-parasite relationships which could be exploited for the purposes of biocontrol. However, any apparent correlation between *Pasteuria* spp. and nematode sequences would require further experimental validation. Preliminary results indicate that this method is appropriate for the sensitive, specific, and semi-quantitative profiling of *Pasteuria* species from eDNA.

CHARACTERISATION OF CLINICAL *CLOSTRIDUIM DIFFICILE* PCR RIBOTYPE 002 ISOLATES FROM DIFFERENT TIME LINEAGES

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Introduction: *Clostridium difficile* infection (CDI) is the major cause of hospital-acquired diarrhoea. Incidence and severity of CDI has increased significantly recently, with varied distribution of PCR ribotypes among countries. Recently, *C. difficile* PCR ribotype 002(CD002) has increased in prevalence in the UK, yet the drivers for this remain unclear. This characterised UK CD002 isolates from different time lineages, and assess phenotypic/genotypic traits that may help explain its emergence.

Methods: Sixty CD002 were studied: UK isolates 2007-2008 (N=15), UK isolates 2011-2013 (19 locations, N=22), and non-UK European isolates 2012-2014 (N=23, 20 locations). Antimicrobial susceptibilities to 16 antimicrobials were assessed using an agar dilution method. The mechanisms of resistance to erythromycin, clindamycin, ciprofloxacin, and moxifloxacin were investigated by amplification and sequencing of resistance genes (*ermB, gyrA & gyrB*). Biofilm formation was quantified using 96-well microtitre plate crystal voilet assays. Biofilm viable counts (spores and vegetative cells) were enumerated after 3 and 6 days (CFU/mL). The maximum specific growth rate (μ_{max}) was measured and the cytotoxin titres ($\log_{10}$ relative units (RU)) were evaluated over a period of 72h using a Vero cell cytotoxicity assay. The total sporulation capacities of CD002 strains were quantified by enumerating spores formed in a liquid media over 120h (CFU/ml) using agar-based culture methods.

Results: All isolates were susceptible to metronidazole, vancomycin, chloramphenicol, linezolid, tetracycline (MICs ≤2mg/L), and resistant ciprofloxacin (MICs ≥8 mg/L). Resistance to clindamycin (16.7%), erythromycin (3.3%), moxifloxacin (1.7%), and nitrofurantoin (1.7%) was present. All but one *C. difficile* isolate demonstrated intermediate resistance to beta-lactam antibiotics (MICs >1mg/L). One UK isolate (2007-8 lineage) was classified as multidrug resistant (MDR). Sequence analysis revealed double substitutions in *gyrA,* Thr-82-Ile and Ala-118-Ser, in two CD002 isolates, while 32 CD002 isolates had only one substitution in *gyrA*, Ala-118-Ser. No amino acid substitutions were found in *gyrB***.** The recent CD002 (UK and non-UK European) strains formed significantly more biofilm *in vitro* than the 2007-8 strains ($p<0.001$). Spore counts within boifilms were significantly greater in recent UK and non-UK CD002 ($P<0.002$). The maximum specific growth rate in non-UK CD002 (2012-2014) strains was significantly higher ($p<0.001$) than that of strains from the UK (2007-2014) regardless of year of isolation. Cytotoxin production overtime did not differ significantly between lineages (median titres 2-3RU). The sporulation rates for the recent isolates were significantly higher ($p<0.001$) than those of the older isolates.

Discussion and Conclusion: Overall, the data generated in this study showed that CD002 from different time lineages did not differ substantially in antimicrobial susceptibility patterns. Additionally, the more recent isolates (UK and non-UK European) formed more extensive biofilms, had higher μ_{max} and sporulated more

rapidly than the 2007-8 isolates. These characteristics were found to be more prominent among strains originating from outside of the UK. The increased sporulation rate and a higher μ_{max} demonstrated by UK CD002 may offer this ribotype a competitive advantage over other ribotypes in the clinical setting and help to explain the recent increased prevalence of this ribotype. However, further genotypic and phenotypic characterisations are required to explain these observations.

ATHENA SWAN LECTURE

Prof. Amrita Ahluwalia,

William Harvey Research Institute, London

Pathway to Silver

Chair: Dr Louise Mackenzie, LMS Athena SWAN Champion

ABSTRACTS OF POSTER COMMUNICATIONS

PHARMACOLOGY

1. NEUROPROTECTIVE EFFECTS OF NOVAL SULPHANAMIDES IN RODENTS' MODEL OF PARKINSON'S DISEASE

Bolouri, L.

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Introduction: Treatment of neurodegenerative diseases is an urgent medical need. Currently there are no treatments that can reverse or even halt the progress of neurodegeneration. Furthermore, the processes involved in neurodegeneration are complex, involving a cascade of events that will ultimately lead to cellular dysfunction and lethality. Inflammation is the major component of all neurodegenerative diseases including Multiple sclerosis, Alzheimer's disease and Parkinson's disease. In post-mortem tissues as well as in animal models of Parkinson's disease, neuroinflammation was shown to be an important component of the neurodegenerative cascade (Barnum & Tansey, 2011). Parkinson's disease is characterized by a slow and progressive degeneration of dopaminergic neurons in the substantia nigra. Despite intensive research, the cause of the neuronal loss in Parkinson's disease is poorly understood. Neuroinflammatory mechanisms might contribute to the cascade of events leading to neuronal degeneration. We describe the evidence for neuroinflammatory processes from post-mortem and in vivo studies in Parkinson's disease. We further identify the cellular and molecular events associated with neuroinflammation that are involved in the degeneration of dopaminergic neurons in animal models of the disease.

Method: Several in vivo studies in animal models have shown that high doses of steroidal as well as nonsteroidal anti-inflammatory drugs significantly reduce neurotoxin (6-hydroxydopamine)-induced pathology in rats. This project will examine the effects of a range of novel anti-inflammatory compounds with good brain penetration in cellular and in rodent models of Parkinson's disease (Hirsch and Hunot, 2012). Therefore, the purpose of this study is to identify and characterize novel anti-inflammatory agents as potential neuroprotective agents. Several investigations have shown that pre-treatment of animal models of Parkinson's disease with anti-inflammatory agents such as steroidal anti-inflammatory agent dexamethasone or cyclooxygenase-1 & 2 inhibitors such acetylsalicylate or meloxicam respectively could be neuroprotective (Teismann and Ferger, 2000). However, these agents cross the blood brain barrier poorly and consequently very large doses would be required. Recently, a series novel arylsulphonamide derivatives with excellent brain penetration and potent anti-inflammatory properties were synthesized (Soskic and Iravani; patent pending). The object of this study is therefore to test the potential role of these compounds in the animal models of Parkinson's disease by stereotactic surgery and primary cell culture method.

Results: Some immunohistochemistry has been examined on ready blocks of different regions of marmoset brains (Mid, forebrain, Hind brain, Prefrontal cortex) administered by +-MPTP and naive which already exist in the laboratory to learn how to use image J software and taking image and quantifying and measuring the amount of DA or Serotonin in particular section. The preliminary findings suggest that our new compound in primary cell culture shows development of neuroprotective effect on cellular level and

vivo.

Conclusion: The project provides a range of *in vitro* and *in vivo* techniques relevant to modern neuropharmacology. Overall, the project provides excellent research training in a laboratory renowned for its expertise in PD and this research will be the cutting edge of current thinking on PD.

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2. EFFECTS OF KETAMINE IN RAT URINARY BLADDER

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Introduction: Ketamine is a non-competitive N-methyl D-aspartate receptor antagonist that has long been used in both human and veterinary medicine as an analgesic and anaesthetic agent. Due partly to its hallucinogenic properties, ketamine is also used as a recreational drug, and its popularity has seen rapid growth in recent years. This has led to the discovery of a link between chronic ketamine use and the development of cystitis and various lower urinary tract (LUT) symptoms. With the clinical use of ketamine expanding to the treatment of depression, and with its current role in pain management, the long term effects of ketamine on the bladder are becoming increasingly important to consider.

Therefore, current research is hoping to advance our understanding of ketamine's mechanism of action in the bladder. Recent findings indicate that ketamine has a direct toxic effect on human urothelial cells and can cause thinning of the urothelium within 72 hours. This impaired urothelial integrity could then lead to leakage of urinary potassium into the underlying tissue, causing many of the LUT symptoms associated with ketamine-induced cystitis. Therefore, the effects of 3mM ketamine on rat bladder tissue will be investigated.

Methods: Rat bladder will be dissected into halves and incubated at 37°C with 3mM ketamine for 72 hours. Tissue will then be embedded in paraffin wax, sectioned and stained with haematoxylin and eosin to assess whether ketamine has any effect on the urothelium or detrusor muscle.

Results: To be presented at conference.

3. MODELLING NON-MOTOR SYMPTOMS OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disease resulting from the loss of dopaminergic neurons in the nigrostriatal tract. Loss of dopamine leads to dysregulation of the activity of the motor circuitry leading to the classical symptoms of rest tremor, instability, hunched posture and slowness of movement. PD is also associated with a constellation of maladies that are debilitating, treatment limiting and affect the quality of life of the PD sufferers, but do not involve movement. Some important non-motor symptoms include, cognitive deficit, deficits in basic sense of taste and olfaction, sleep disturbance and dysregulation of the autonomic system. These non-motor symptoms are thought to be unrelated to the loss of dopamine, as dopamine replacement therapies that improve motor deficits have little or no effect in reversing them.

Non-motor symptoms of PD have historically been under-recognised, but are now being given more importance, highlighted by the recent MDS clinical diagnostic criteria for PD, which puts a 'red flag' against a diagnosis of PD if non-motor features are absent by 5 years of disease duration (Postuma, Berg et al. 2015). Autonomic dysfunction, mainly those associated with gastrointestinal (GI) problems, often precede the onset of motor symptoms by many years. The GI dysfunction can be seen at all levels of the GI tract, and almost all patients suffer from impairment at some point. The most frequent symptoms include constipation, gastroparesis and dysphagia. Our aim is to study the functional and biochemical aspects of the GI function in various rodent models, to discover pathways by which such issues may arise at local and central levels.

Methods: *In vitro* organ bath bioassays will form the core of our experiments for determining the functional aspects of GI symptoms in PD models, of nigrostriatal degeneration. Peristaltic activity will be measured on segments of the GI tract and compared to the motor activity of control tissue (Keating, et al. 2014). The GI tract is richly innervated with extrinsic sensory afferents that provide the basis for reflexes, behavioural responses and sensation. These nerves project the sensory signal to the spinal cord and in turn to higher brain regions. Using multiunit recording of afferent nerve discharge we will examine whether GI dysfunction in PD is due to the impairment of sensory signalling to the CNS.
To complement this, we will examine histopathological, neurochemical and genetic changes within these models to elucidate more intricate mechanisms by which such impairment occurs. Techniques to be used will include immunohistochemical staining, RT-PCR, qPCR and Western blot analyses.

Conclusion: A multimodal systematic approach is necessary to fully characterize GI dysfunction in PD. This work will examine the temporal and spatial relationship between the loss of dopaminergic neurons and the onset of functional changes in the GI tract. The ultimate aim is to gain a better understanding not only of GI dysfunction but PD as a whole, leading to effective early intervention and better quality of life.

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4. EFFECTS OF *PIPER SARMENTOSUM* AND *TINOSPORA CRISPA* ON VASCULAR TONE AND ON NITRIC OXIDE PRODUCTION

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Introduction: Contraction and relaxation of arteries (vascular tone) are vital for the regulation of blood flow and/or pressure and is regulated by several intrinsic and extrinsic factors. Nitric oxide (NO) and underlying oxidative stress (OS) play an important role in the pathogenesis of diabetes, atherosclerosis, and/or hypertension. Herbal medicinal plants provide alternate therapy in these pathologies. Leaves of *Piper Sarmentosum* (*PS*) and *Tinospora Crispa* (*TC*) are widely used as traditional medicine, for anti-diabetic properties. However, whether these plants regulate vascular tone remains to be investigated. Therefore, we aimed to investigate the effects of cold ethanolic extracts on vascular tone of rat aortic strips and further extended to see their direct effect on NO production and on OS *in vitro* in rat cultured aortic smooth muscle cells (RASMCs).

Methods: The aorta was collected from male Wistar rats (weighing 300-450g) and a strip of ~1.5mm was suspended in an organ bath. Dried and powdered plant leaves were then extracted in cold ethanol (90%) followed by evaporation at 40°C and freeze drying. Contractile dose response curves (DRCs) to phenylephrine (PE; 1pM-30µM) were constructed and EC_{80} of PE was used to contract tissues in further experiments looking at relaxations to acetylcholine (ACh; 1nm-30µM). In parallel, aortic strips were pre-incubated for 1 hr with OS inducers alone such as homocysteine (Hcy; 1&100µM), antimycin A (AA; 1&100µM) or co-incubated with *PS* (1µg ml^{-1}) or *TC* (1µg ml^{-1}) to investigate modulation of vascular tone. Effects to both plant extracts (1-100 1µg ml^{-1}) were also investigated with or without AA (50µM) in cells stimulated with bacterial lipopolysaccharides (LPS; 100µM^{-1}) and interferon-γ (IFN-γ; 100U ml^{-1}). Cell viability, NO production, protein concentration was assessed by the MTT, Greiss and BCA assay respectively.

Results: Contractions of aortic strips induced by PE were increased significantly ($p<0.05$) in the presence of 1µg ml^{-1} of PS, but reduced by ~20% when pre-incubated for 1hr with Hcy (1µM; $p<0.05$). Extract of PS (1µg ml^{-1}) were unable to restore Hcy mediated inhibited contractions, while TC (1µg ml^{-1}) was able to reverse the effects ($p<0.05$). Similarly, ACh induced relaxations were inhibited by ~30% upon pre-incubation with Hcy (1µM; $p<0.05$) and restored marginally by PS but not with TC at 1µg ml^{-1}. Contractile effect of PE were not altered in the presence AA (1µM), however reduced to ~50% with both PS ($p<0.01$) and TC ($p<0.05$) at 1µg ml^{-1}. ACh induced relaxation were completely abolished by AA (1µM; $p<0.01$). The inhibition was not affected by PS or TC. Both PS and TC (1-100µg ml^{-1}) did not induced nitrite production in RASMCs. However, both extracts increased nitrite production by ~20-40% in a dose dependent manner at concentration ranging from 1-50µg ml^{-1}; and inhibited by ~50% at 100µg ml^{-1}. In addition, PS and TC were able to partially restore nitrite production. MTT data reflected no significant toxicity in cells incubated with drugs and/or plant extracts.

Conclusion: These preliminary data suggest that both PS and TC regulate vascular tone aortic strips *in vitro*. Cell culture data suggest a biphasic response of plant extracts on nitrite production. Future research work will explore expression of inducible nitric oxide synthase in experimental condition and mechanisms associated with regulation of oxidative stress signalling pathways.

5. LYSOPHOSPHATIDIC ACID (LPA) INDUCED EXPRESSION OF MIR-145 AND MIR-1 IN CARDIOMYOCYTES DERIVED FROM P19 STEM CELLS

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Introduction: MicroRNAs (miRNAs) are a group of small non-coding RNAs that play a crucial role in several pathologies through regulation of post-transcriptional activation of genes. LPA is a circulating bioactive phospholipid which is involved in cellular processes like proliferation, apoptosis inhibition amongst other actions. Elevated levels of LPA have been reported in myocardial-infarction while lower levels may be involved in regulating the differentiation process post-transcriptionally. Studies were conducted to determine the mechanism of LPA induced differentiation of P19 stem cells into cardiomyocytes and further establish whether this may be associated with changes in miRNA expression.

Method*:* A teratocarcinoma P19 stem cell line was cultured in complete medium (α-MEM) and plated in non-adherent Petri dishes for 4 days to form embryoid bodies (EBs). Subsequently, these EBs were transferred into adherent tissue culture plates. Cells were incubated with 5 µM LPA during EBs formation stage and lysates were collected on day 0-12. In parallel, cells were incubated with LPA at 0.5-20 µM and lysates collected on day 6. This Differentiation of EBs was confirmed by visualisation of beating cells. Western blotting for ventricular myosin light chain (MLC-1v) was detected. Expression of other markers such as OCT4 were also determined. Image-J software was used for semi-quantification. Furthermore, RT-PCR for mir-145, mir-1 and OCT-4 were investigated in cardiomyocytes and P19 cells.

Results: It was confirmed that LPA was involved in the differentiation of P19 cells into a cardiac lineage in a time and concentration dependent manner. An elevation in expression of MLC-1v against the increase in concentration of LPA was evident ($p<0.01$) this was supported by the observation of beating cells. Expression of OCT4 decreased significantly in parallel with increase in expression of cardiac specific markers. Furthermore, mir-145 was found to be significantly up-regulated by 2.043 folds in induced cardiomyocytes by 5 µM of LPA. Same concentration of LPA showed small but significant elevation on the expression of mir-1 at day 6.

Conclusion: LPA in its physiological levels (2-20 µM) is established to induce differentiation of P19 stem cells in a dose and time dependent manner. Additionally, mir-145 & mir-1 are regulated by LPA in induced cardiomyocytes. These findings suggest that endogenous factors like LPA could be exploited in future therapeutic strategies.

6. EFFECTS OF ASPIRIN AND SALICYLIC ACID ON LPA INDUCED DIFFERENTIATION OF P19 STEM CELLS INTO CARDIOMYOCYTES

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Introduction: Myocardial infarction (MI) is the leading cause of the increased cardiovascular disease mortality in people around the world. In recent years, there has been a concerted effort in exploiting stem cells based cardiac therapies for myocardial regeneration. Aspirin is widely used to reduce the risk of cardiovascular relate events including MI. Other studies have suggested that aspirin regulates stem cell proliferation. These observations indicate that aspirin may be of some benefit in facilitating the differentiation of stem cells. Previous research in our group established that a physiological phospholipid lysophosphatidic acid (LPA) regulates cell growth and differentiation of P19 mouse embryonic teratocarcinoma stem cells into cardiomyocytes. Therefore, we aimed to investigate the effect of aspirin and salicylic acid (SA) on the differentiation of P19 stem cells into cardiomyocytes in the presence or absence of LPA and underlying signalling pathways involved in this process.

Methods: The P19 mouse embryonic carcinoma stem cells will be used in all studies as this cell line has already been characterized by our group. The cells will be cultured in alpha minimal essential medium supplemented with 10% foetal bovine serum, penicillin (100U ml^{-1}) and streptomycin (100µg ml^{-1}). Embryoid bodies (EBs) will be formed in non-adherent sterile Petri dishes in the presence of the treatment conditions for 4 days followed by transfer into 6 well plates and cultured for 6 or 12 days in a monolayer form. Both the aspirin (0.01-5 mM) and SA (0.01-5 mM) were pre-incubated for an hour prior to the addition of LPA (5µM). Cells will be also incubated with 1% DMSO in parallel as a positive control. At the end of each incubation period, cold cellular lysis was performed and protein concentration was assessed using bicinchoninic acid (BCA) assay. Cell lysates were then probed for expression of the cardiac specific myosin light chain-1v (MLC-1v) by western blotting. Cellular toxicity was also investigated using MTT assay in identical experimental conditions.

Results: Both the aspirin and SA induced significantly higher toxicity ($p<0.01$) at 5mM and 10mM. Toxic effects of aspirin and SA were similar when incubated with 5µM of LPA and did not differ statistically. Also, LPA (1-25µM) did not induce any significant toxicity and viability was very similar of control cells. Preliminary data suggest that DMSO (1% v/v) and LPA (5µM) induce differentiation into cardiomyocytes. Also, both the aspirin and SA did not induce differentiation without LPA. Future research work will repeat these experiments to confirm these findings.

Further work: Establish studies to investigate the concentration and time dependent effects of LPA on the differentiation process. Initiate studies looking at the concentration dependent effects of aspirin and salicylic acid on cell viability and on differentiation of P19 cells in the absence and presence of LPA. Initiate studies on signalling pathways regulated by LPA selecting targets from data of ongoing studies in the group. This will include key signalling pathways including the MAPKs and PI3K which have been shown to be critical

for differentiation. Investigate whether aspirin and/or salicylic acid regulate differentiation through these pathways.

7. EFFECT OF ORAL PI3KINASE DELTA INHIBITION AND LEUKOTRIENE D4 ANTAGONISM IN A MURINE MODEL OF HDM-INDUCED PULMONARY INFLAMMATION

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Introduction: Despite much research in the asthma field, inhaled corticosteroid (ICS) therapies are a mainstay of anti-inflammatory treatment for asthma. Montelukast (a cysteinyl leukotriene antagonist) is used as an alternative anti-inflammatory approach in the treatment of asthma (Janeva, Goseva, Gjorchev *et al.*, 2015; Ciolkowski, Mazurek, Hydzik *et al.*, 2016). Phosphatidylinositol 3-kinase delta (PI3Kδ) inhibitors have been reported as a potential new line of experimental non-steroidal anti-inflammatory agents in the treatment of asthma (Rowan, Smith, Affleck and Amour, 2012; Sriskantharaja, Hamblin, Worsley *et al*, 2013; Southworth, Plumb, Gupta *et al.*, 2016). The aim of the present study was to compare the efficacy of montelukast and a PI3Kδ inhibitor in a model of HDM-induced allergic pulmonary inflammation.

Methods: Under isofluorane-induced anaesthesia, female BALB/c mice were intra-nasally challenged once a day for 5 days a week over a 3 week period with House Dust Mite (HDM) extract. The inflammatory response in the lungs was then allowed to partially resolve for a period of 2 weeks. Mice were then intra-nasally re-challenged with HDM. The anti-inflammatory effects of an established leukotriene D4 antagonist (montelukast) and a novel oral PI3kinase delta inhibitor (Compound 'X') were assessed by dosing 7 days prior to HDM re-challenge and up to cull time-points. Mice were then sacrificed at pre-determined time-points to assess effect on; cytokines (4hrs post HDM re-challenge), eosinophils and neutrophils (24hrs post HDM re-challenge) and lymphocytes (72hrs post HDM re-challenge).

Results: The PI3kδ inhibitor, dose-dependently inhibited both BAL and serum cytokines at the relevant optimum time-points for representative cytokines. However, montelukast was only able to significantly inhibit serum IL-2 and KC. Eosinophil infiltration in both BAL and lung was dose-dependently inhibited by the PI3kδ inhibitor. Neutrophil inhibition with the PI3kδ inhibitor was only observed in the lung at the 10mg/kg dose. PI3kδ inhibition also exhibited significant reductions in BAL and lung lymphocyte subset infiltration. Montelukast did not significantly inhibit infiltration of any BAL or lung cell type.

Conclusions: Montelukast and steroids are currently being used as therapies for the treatment of asthma. In the mouse model of HDM-induced allergic pulmonary inflammation described in the present series of studies, a novel anti-inflammatory approach by way of PI3kδ inhibition was shown to be better at inhibiting pulmonary inflammation compared to inhibition of leukotriene D4 antagonism alone using montelukast.

POSTGRADUATE MEDICINE

8. FACIAL RECOGNITION ABILITIES: AN INFERENTIAL QUASI-EXPERIMENTAL STUDY IN IRAQI MEDICAL STUDENTS

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Introduction: The ability of modern-day humans to recognise faces of countless individuals is unique and has an evolutionary basis (Davis et al., 2016). The cortical surface area dedicated to this task is significantly large. This aim of this study is to analyse the facial recognition abilities of selected Iraqi individuals and to correlate these abilities with gender, Handedness, and ethnicity (Al-Hadithi at al., 2016)

Methods: The study was initiated in October 2016; it is quasi-experimental and cross-sectional in design. Participants were medical students (n=309) aged 17 to 25 years, and of four ethnic groups: Arabic (288), Kurdish (12), Turkish (7), and Christian ethnicities (2). The facial recognition ability was numerically scored (0-14) using a facial recognition test. The test was distributed electronically via the university's intranet. Non-parametric inferential statistics were used to correlate each score with sex, handedness, and ethnicity.

Results: There was a statistically significant difference between males and females (*p-value* of 0.027). There were no statistically significant differences in between right-handed and left-handed individuals. There was an inter-ethnic significant difference in between Arabs and Kurds with (*p-value* of 0.022).

Conclusions: Facial recognition abilities were never investigated before in the Iraqi population. This study has proven the presence of a correlation between gender and facial recognition, ethnicity and facial recognition. Individuals with high scores on facial recognition tests are known as super-recognizers. Super-recognizers are "precious" to be employed in law-enforcement and intelligence agencies worldwide. Practical applications of this study are not limited to biometrics, artificial intelligence, and anthropometrics (Smeets et al., 2010).

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9. EXERCISE ADDICTION AND THE USE OF ENHANCEMENT DRUGS IN FITNESS CLUBS IN THE UK: A PILOT STUDY

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Objective: Physical exercise has increasingly been viewed as potentially addictive. The aim of this study was to explore exercise addiction and the use of Performance and Image Enhancing Drugs (PIEDs) alongside other products to either lose weight or achieve fitness goals in gym settings.

Method: A questionnaire was disseminated both online and face-to-face among members of fitness clubs in the United Kingdom.

Results: Although an interest in physical activities was expected from a population recruited in fitness settings, a prevalence of individuals affected by or at risk of exercise addition emerged from our study (15% of the sample). 377 questionnaires were completed. Almost half (48%) of the participants disclosed PIEDs use to either lose weight or help them reach their fitness goals. 21% experienced side-effects, such as palpitations, change in mood, acne and gastrointestinal effects. The Internet played a major role in both the supply of information and the provision of PIEDs, which adds the risks of contamination and the consumption of undisclosed ingredients. Low self-esteem, exercise addiction and appearance anxiety were seen as potential motivations for PIEDs use.

Conclusion: Exercise addiction as well as the diffusion of PIEDS to enhance fitness goals or lose weight in fitness contexts are rapidly expanding phenomena, which remain poorly investigated. Further assessment will also need to be carried out on this topic, which will be my main task during my Visiting Fellowship at the University of Hertfordshire.

10. OPIATE USE IS ASSOCIATED WITH REDUCED ANTIPLATELET DRUG EFFECT AND RAISED TROPONIN IN PPCI

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Background: The emergency management of ST-elevation myocardial infarction (STEMI) involves dual antiplatelet therapy (DAPT) administration and primary percutaneous coronary intervention (PPCI). Pain is treated with opiates, which may delay gastric transit, and reduce DAPT absorption. We sought to assess the effect of morphine on reperfusion, infarct size and thrombotic status.

Methods: 300 patients presenting for PPCI were assessed for ST-segment resolution, coronary flow, thrombotic status and peak troponin. Morphine was given as required by emergency teams *en route* to the heart attack centre. All patients received DAPT and PPCI was performed according to standard care, with optional glycoprotein IIb/IIIa inhibitor (GPI) use.

Results: Patients receiving morphine (n=218; 72.7%) had significantly less spontaneous ST-segment resolution pre-PPCI, lower rate of TIMI 2/3 flow in the infarct-related artery pre-PPCI, and higher troponin level post-PPCI (1906[1002-4398] vs. 1268[249-2920]; p=0.016) than those who did not. Patients receiving morphine exhibited significantly enhanced platelet reactivity and impaired endogenous fibrinolysis on arrival, compared to no-morphine patients. Morphine administration was an independent negative predictor of spontaneous ST-segment resolution after adjustment for other variables (OR 0.26; CI 0.08-0.84; p=0.025). Among patients receiving GPI, there was no difference in pre-PPCI flow or peak troponin according to morphine use, indicating that the adverse effects of morphine relate to delayed DAPT absorption that is overcome by GPI.

Conclusions: Morphine use in STEMI is associated with enhanced platelet reactivity, reduced spontaneous reperfusion and larger infarct size, and these adverse effects may be overcome by GPI use.

Clinical Trial Registration: http://www.clinicaltrials.gov. Unique identifier: NCT02562690

11. COMPARATIVE EFFECTS OF DIFFERENT P2Y12 INHIBITORS ON GLOBAL THROMBOTIC STATUS IN CORONARY ARTERY DISEASE

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Background: Patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI) are at risk of thrombosis. Enhanced platelet reactivity and impaired endogenous thrombolysis, despite dual antiplatelet therapy, are risk factors for recurrent thrombotic events. We aimed to compare the relative effects of different P2Y12 antagonists on global thrombotic status including endogenous fibrinolysis.

Methods: Patients with established CAD taking aspirin 75 mg daily and scheduled to undergo PCI, were tested for thrombotic status before and after being established on clopidogrel (n=20), ticagrelor (n=20) or cangrelor (n=15). Thrombotic status was assessed with the automated point-of-care Global Thrombosis Test (GTT, Thromboquest Ltd., UK) which utilizes native, non-anticoagulated blood to assess in vitro occlusive thrombus formation (occlusion time OT, sec) and the time taken to restore flow through endogenous thrombolysis of the occlusive thrombus (lysis time LT, sec). Patients were tested after at least 7 days of continuous oral P2Y12 inhibitor or 5-7 min following the start intravenous cangrelor infusion.

Results: All P2Y12 inhibitors significantly prolonged OT compared to baseline, with the magnitude of effect greatest for cangrelor, followed by ticagrelor, then clopidogrel (mean increase in OT from baseline (delta): clopidogrel 83±120 vs. ticagrelor 102±112 vs. cangrelor 234±213; p=0.01). Cangrelor was the only P2Y12 inhibitor to significantly reduce LT compared to baseline. The difference in LT when comparing samples pre and post P2Y12 inhibition was significantly different between the 3 groups (median change in LT (delta) from baseline: clopidogrel 30 [IQR-323, 260] vs. ticagrelol 81 [-58, 259] vs. cangrelor 309 [111, 764], p=0.041).

Conclusion: All P2Y12 inhibitors reduced platelet reactivity as evidenced by increase in OT, with magnitude of effect being cangrelor > ticagrelor > clopidogrel. Of all P2Y12 inhibitors, only cangrelor favourably enhanced endogenous thrombolysis. Further studies are needed to see if preferential cangrelor use in patients with impaired endogenous thrombolysis may translate into improved clinical outcomes.

Keywords: P2Y12 receptor antagonists, thrombosis, fibrinolysis, coronary artery disease

PHARMACY

12. MEDICINE RELATED PROBLEMS IN ADULT PATIENTS WITH ATRIAL FIBRILLATION ON DIRECT ORAL ANTICOAGULANTS: A RETROSPECTIVE STUDY

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Introduction: Atrial fibrillation (AF) is the most common type of cardiac arrhythmia, affecting approximately 1.5–2% of the population worldwide (Camm et al. 2012). Presence of AF is associated with a five times higher risk of stroke, three times higher risk of heart failure and increased mortality (Camm et al. 2012). Anticoagulants are recommended to prevent stroke and systemic embolism in AF and are a significant cause of patient safety incidents (Perzborn et al., 2011). Recently, direct oral anticoagulants (DOACs) have been developed as alternatives to traditional anticoagulant such heparin and warfarin. The numbers of patients using DOACs have continued to increase over time (Romanelli *et al.,* 2016). Although clinical trials data shows a similar adverse drug reaction profile of DOACs and Warfarin, real world observational evidences on Medicine Related Problems (MRPs) for DOACs are lacking. Therefore, we aim to investigate medicine related problems associated with DOACs in adult patients with Atrial Fibrillation (AF).

Methods: A retrospective review of patients' medical records (admitted between November 2015 to April 2016; last admission) was reviewed at Lister Hospital in Stevenage East and North Herts NHS Trust in the U.K. The data collection included; patients' demographic information, diagnosis, risk factors, details of the case (such as chief complain, vital sign, assessment, and management plan), medication history, laboratory tests and results. MRPs were defined and classified based on Pharmaceutical Care Network Europe (PCNE V6.2). MRPs identified were validated by the researcher and a cardiovascular clinical pharmacist in the hospital. Clinically relevant quantitative data from these records were extracted using a data collection form and analysed using SPSS version 22.0.

Results: Data were collected between November 2016 and January 2017. A total of 54 patient records were reviewed. An MRP was identified in 43 patient records 79.6% (n=87). MRPs associated with DOACs were 71.3% (n=62). Medication error (46%, 40 out of 87) and adverse drug reaction (30%, 26 out of 87) were the dominant MRP categories, while bleeding (73%, 19 out of 26), and prescribing errors (60%, 24 out of 40) were frequent subcategories for MRPs.

Conclusion: Medicine related problems associated with DOACs were significant. Most of MRPs are medication errors and adverse drug reactions. The planned total number of patient records to be reviewed is 260. Although initial finding have shown a high percentage of MRPs, further case reviews may alter the current findings.

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13. A SYSTEMS APPROACH TO TACKLING OBESITY IN THE SAUDI CONTEXT

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Introduction: Childhood obesity is widely considered a critical public health issue. In Saudi Arabia, at least 30% of adults, 20% of adolescents and 10% of children are suffering from obesity. The seminal Foresight Report (2007) conceptualised obesity as a complex, systematic, and multi-causal problem. To account for this complexity, a systems approach is desirable, which recognises composite sub-systems and the feedback loops and can help to create effective solutions. The systems map must be informed by qualitative and quantitative data relevant to the local context, and must be implementable. This study aims to develop a systems map of current causal factors and interventions in the context of Saudi Arabia to address unhealthy weight in children.

Methods: System dynamics (SD) is used as a guiding methodological approach. This approach is emerging in the area of public health but has not been applied to obesity problem in the Gulf region. This study is comprised of four phases. **1**: Identification of national and international policies that deal with obesity by reviewing the literature on currently implemented interventions that could be adapted to the Saudi context. Additionally, we conducted direct observation on an integrated obesity care pathway programme in England (Lambeth healthy weight pathway for children / (CHALK)) and face to face semi-structured interviews were conducted with key people and stakeholders from multiple organisations in the Saudi context. **2**: Investigation of factors in schools' environment that could influence children's healthy eating and physical activity behaviour; we conducted a mapping of availability and density of fast food outlets and convenience stores located within 0.5 km^2 of primary schools in the city of Narran in Saudi Arabia. **3:** Assessment of parents' and teachers' knowledge, behaviours and attitude that could influence the healthy eating and physical activity of children; two surveys were conducted in Najran using questionnaires: one targeted parents (787 respondents) and one targeted teachers (336 respondents). **4:** Influence diagrams and causal loop diagrams are being developed using the quantitative and qualitative data extracted from phases 1, 2, and 3.

Results: Despite the efforts of addressing health promotion in childcare settings and schools aligned with national awareness campaigns, implementation of interventions remains ad-hoc, relying on local resources which vary across regions. The interviews with stakeholders examined qualitative factors that influence the implementation of childhood obesity prevention policies. It was observed that positive factors included good provision of health facilities, existence of obesity coordinator post and noncommunicable diseases department, and awareness campaigns in public places. Whereas, barriers included serving unhealthy food in schools, lack of playgrounds and sports facilities in schools, and high consumption of energy drinks and unhealthy snacks. However, these initial findings need to be supported with more results from the surveys

to inform the process of building the influence and causal loop diagrams of childhood obesity in the Saudi context.

Conclusion: This study will provide the first system map related to obesity in the Saudi Arabia by focusing on the school environment for primary school children aged 6-12 years old.

14. IDENTIFICATION AND VALIDATION OF MODE OF ACTION OF CHALCONES AS ANTI-TUBERCULAR COMPOUNDS

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Background: Tuberculosis (TB) is the most devastating infectious disease caused by the *Mycobacterium tuberculosis* (MTB) bacillus existed for millennia and remains a global health problem. Poor compliance is partly to blame for the evolution of drug-resistant MTB strains that are difficult and expensive to treat. The treatment for RIF-resistant TB, multidrug-resistant TB, and extensively drug-resistant TB is even longer (18-24 months), and requires more expensive and more toxic drugs. The emerging problem of antimicrobial resistance is proving to be a bigger challenge in the post-antibiotic era and the search for new drugs has become one of the great challenges for medicinal chemistry. An improvement in the outcomes of TB chemotherapy can be achieved by the development of new, shorter, cheap, safe and effective anti-TB regimens. In this context, we have explored chalcones as potential anti-tubercular compounds. Chalcones, being natural or synthetic compounds are known to display a remarkable spectrum of biological activities such as antibacterial, anti-malarial, anti-inflammatory, analgesic and as antioxidants. They are also well known as valuable intermediates in organic synthesis of many heterocyclic compounds that exhibit a multitude of biological activities.

Method: Chalcones (**1a**-**1o**) were synthesized by reacting aromatic-aldehydes with various acetophenones by the Claisen-Schimidt condensation in the presence of sodium hydroxide in ethanol. The synthesized products were recrystallized from appropriate solvents and were characterized by spectral analysis, melting point, infrared spectroscopy, ^{1}H and ^{13}C NMR and mass spectrometry. Minimum inhibitory concentrations (MIC) were determined for these synthesized compounds by broth micro-dilution according to CLSI guidelines. Based on the Selectivity Index (SI) values obtained from cytotoxic studies performed on mouse macrophage J774 cell line, compound **1a** (SI=8.4) was selected for mode of action elucidation.

To investigate whether **1a** affects the synthesis of mycobacterial lipids, mycolic acid methyl esters (MAMEs) and fatty acid methyl esters (FAMEs) were extracted and analyzed by TLC. A dose dependent reduction of MAMEs with the overall abundance of FAMEs suggests that **1a** targets mycolic acid biosynthesis (fatty acid synthase (FAS)-II inhibitors). To further corroborate **1a** inhibits mycolic acid biosynthesis, the impact on the MIC was investigated using strains of *M. bovis* BCG over-expressing components of FAS-II. The ample growth of InhA over-expressor strain was observed, indicating an increase in resistance and the MIC shift of > 4X providing further evidence to support InhA as the cellular target for **1a**.

Table1: *In Vitro* preliminary screening data of Chalcones									
Compound	Substituents			M.wt	C*logp*	MIC (µg/ml)		J-774 (IC50)	SI=IC50/MIC
	R_1	R_2	R_3			m.smegmatis	m.bovis		
1a	H	H	H	270.33	3.35	50	6	50	8.4
1b	H	Br	H	349.22	4.18	-	-	25	-
1c	Br	H	H	349.22	4.18	100	25	25	1
1d	H	H	Br	349.22	4.18	200	12	25	2.08
1e	H	Cl	H	304.77	3.91	-	-	12	-
1f	Cl	H	H	304.77	3.91	100	12	12	1
1g	H	H	Cl	304.77	3.91	-	200	12	0.06
1h	H	H	F	288.32	3.51	200	25	100	4
1i	H	H	OCH_3	300.35	3.23	200	50	100	2
1j	H	H	CH_3	284.35	3.84	100	25	25	1
1k	H	H	C_6H_5	346.43	5.03	200	100	25	0.25
1l	H	NO_2	H	315.32	3.48	-	100	25	0.25
1m	CH_3	H	H	284.35	3.84	200	50	25	0.5
1n	H	H	NO_2	315.32	3.48	-	200	50	0.25
1o	Cl	H	Cl	339.21	4.47	-	200	6	0.03

15. SOLID PHASE MICROEXTRACTION (SPME): *IN VIVO* DRUG DETECTION IN TISSUES AND ORGANS

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Introduction: Drug development requires preclinical testing whereby compounds are dosed in animal models to assess the tissue distribution of developmental compounds. Drug distribution analysis commonly requires animal sacrifice, organ homogenisation, and subsequent analysis; a sampling protocol that is inherently costly in terms of animal life. Solid Phase MicroExtraction (SPME) is an equilibrium technique for sampling drug molecules in complex matrices, which has been previously applied to quantitation of drug in blood. Since SPME is minimally-invasive, the technique allows for sampling *in vivo,* avoiding animal sacrifice. The aim of this project is to examine the potential of SPME for detection and quantification of drugs and metabolites in whole organ tissues.

Methods and Results: LC-UV and LC-MS methods were developed and subject to validation. The final method is as follows: 95% acidified water/5% MeCN to 70% water over 3 min was employed (C18 column 2.1 mm x 1.8 mm, 1.7 µm, at 35˚C, flow rate 0.5 mL min^{-1}, 1 µL injection volume, monitoring at 223 nm or *m/z* transition *268 >> 116*). These were used in order to investigate extraction of a model drug (metoprolol) from a variety of matrices by SPME in comparison to existing extraction methods (Liquid-Liquid Extraction, Solid Phase Extraction). The influence of agitation methods on drug adsorption to and desorption from SPME fibres was examined. Subsequently, drug extraction from a variety of spiked aqueous and lipid matrices simulating organ tissues was performed to select the most suitable fibre for 'in-tissue' studies. For example, the extent of drug extraction from a near-saturated metoprolol phosphate buffered saline (PBS) solution as simulant of interstitial fluid was studied using fibres coated with C18, PolyDimethylSiloxane/DiVinylBenzene (PDMS/DVB), PolyDimethylSiloxane/Carboxen (CAR/PDMS), PolyDiMethylSiloxane/DiVinyl- Benzene/Carboxen (CAR/PDMS/DVB). Total drug extraction was broadly similar for all fibres, but inter-fibre variability was lowest for C18 SPME fibres; C18 extracted (4.3 µg ± 0.05 µg), PDMS/DVB (4.4 µg ± 0.33), CAR/PDMS/DVB (5.03 µg ± 0.25), and CAR/PDMS (5.2 µg ± 0.9). On the basis of the lipid and aqueous screens, the optimum fibres (C18), were then applied to the extraction of metoprolol from skin and liver tissue homogenates.

Conclusions and Future Work: Initial work suggested C18 as the optimal fibre type for metoprolol extraction from both model matrices (PBS, corn oil). This may be reduced as the technique is further developed by use of appropriate SPME-based calibration. Ongoing work will investigate extraction of metoprolol and additional model compounds from homogenised tissue matrices. Future work will employ unhomogenised tissue and later, sampling within organs *in vivo*; by use of perfused systems and live animals.

Acknowledgements and Funding: The authors would like to thank GSK for experimental assistance, and the NC3Rs for funding (Grant NC/L001683/1).

16. IDENTIFICATION OF NOVEL SYNTHETIC CANNABINOID LIKE MOLECULES USING PHARMACOPHORE MODELS

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Introduction: Pharmacophore approaches have rapidly become a common place tool in the discovery of new drug-like molecules. A ligand-based pharmacophore is an abstract representation of what key features of a ligand are crucial to receptor binding in terms of the types of molecular feature and spatial arrangement. Pharmacophores can be generated using ligands that are known to be active as templates, which can be aligned against one another to produce a consensus based model. These models are exceptionally useful and efficient for virtual high-throughput screening of molecular databases that contain millions of compounds. The aim of this study is to generate pharmacophore models based on known synthetic cannabinoids in attempt to discover a novel cannabinoid scaffold that may be exploited in the future.

Methods: A database of 162 known synthetic cannabinoids were clustered into groups using the clustering application in JChemAxon[1] based on similarities in their chemical structures. Pharmacophore model generation for each group was conducted by flexibly aligning the structurally similar cannabinoid molecules. A consensus model was then created based on the aligned motifs. The models were then refined using the Leave One Out (LOO) process, which involves the iterative virtual screening of a known database of 600 Novel Psychoactive Substances (NPS) then altering the model to achieve the best possible retrieval of desirable compounds or “hits” from the Zinc database.

Results: From the clustering process the 3 main synthetic cannabinoid subgroups were the carboxyamide derivatives, carboxyindole derivatives and the napthoylindoles. Due to the structural dissimilarity within the carboxyamide group it was decided to further cluster this group which gave rise to 2 smaller clusters of cannabinoids. The refined models were then used to virtually screen the Zinc database which contained approximately 17 million drug-like compounds. The hits retrieved were then filtered using Log PS (permeability surface area) values which were calculated using the equation:

Log PS = -2.19 + 0.262 * h_LogD + 0.0683 * vsa_base – 0.009 * TPSA[2]

Equation 1: The equation used to calculate Log PS values.

Log PS was selected due to its strong correlation to Log BB (blood brain barrier permeability). The carboxyamide cluster 1 retrieved 271169 hit compounds which is 1.51% of the Zinc database, carboxyamide cluster 2, carboxyindole and napthoylindoles retrieved 833779 (0.47%), 158892 (0.89%) and 121832 (0.68%) hit compounds respectively.

Discussion and Conclusions: All 4 models were able to retrieve between 20-40% of desired cannabinoid molecules in the first 20% of the molecules in an NPS database. This suggests that the pharmacophores

are discriminative and can be carried forwards into virtual screening studies. When the virtual screen was carried out using the Zinc database[3] the number of hits retrieved varied from 0.47-1.51%. Notably the cluster 2 carboxyamide pharmacophore model generated that largest number of hits, when initial refinements of this 7 feature model was being conducted the removal of any features caused the model to loses specificity in identifying the desired synthetic cannabinoids. As the model has mainly aromatic hydrophobic regions this may account for a large number of hits being retrieved from the Zinc database. Additional filters such as aqueous solubility will be applied to further refine the list of hits retrieved.

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17. DEVELOPMENT OF A ROBUST REPRODUCIBLE *IN VITRO* TESTING SYSTEM FOR ENVIRONMENTAL AND PHARMACEUTICAL PARTICLES

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Introduction: Inhalation is now being considered as a potential route for pharmaceutical therapies and is also considered the most important exposure route in humans to environmental particles. Currently there are no reproducible robust *in vitro* models to adequately test both environmental and pharmaceutical particles for biological interaction, bioavailability and toxicological responses. *In vitro* toxicology tests for environmental particles involve cell viability tests as standard. However, nanoparticles can interact with either the components of the assays or the detection method thus giving false positive/negative results. Therefore it is important for particles to be evaluated to ensure compatibility with toxicity assays before being used *in vitro*. The aim of these studies was to determine the evaporation of PBS at different flow rates and volumes using the TSI and NGI, and to compare 4 different assays with different nanoparticles. This data will then enable us to determine if the NGI and TSI are suitable models to use with the A549 cell line as an *in vitro* deposition system and what assays we should use with the nanoparticles we want to test.

Methods: Transwells and Snapwells were loaded with 50 and 100 µL of PBS respectively and selected airflow and volumes were passed through the TSI and NGI. The Transwells and Snapwells were weighed before and after the airflow had passed through the systems. The compounds were suspended in complete cell culture medium (DMEM) at a concentration of 10 mg/mL on the day of experiment. From this a concentration curve was pipetted into a 96 well plate using a 1:2 dilution. Thus giving a concentration range of 10 mg/mL to 0.004883 mg/mL. All particle concentrations were added in triplicate. Assays were then added to the particles following the developed protocols.

Results: Results show the comparison of the TSI, NGI position 2 and 5 showing a significant difference ($p < 0.05$) from 30 L/min for the TSI but no difference for either position of the NGI. A comparison of all the assays tested with a selection of titanium dioxide, silicon carbide and DQ12 particles. There are no significant differences at the lower doses for the MTT, WST-1 and MTS assays, but there are for PrestoBlue ($p < 0.05$).

Discussion and Conclusions: From the results using the TSI we can see that from a flow rate of 30 L upwards there is a sharp decrease in the amount of PBS remaining in the Transwell. If using a flow rate of 30 L or higher this method would not be advisable to use based on these results. The results from the NGI however, showed only a slight loss across all the flow rates with >85% of the PBS remaining in the Snapwells. The MTT, WST-1 and MTS assays should not be used in *in vitro* toxicity testing of these nanoparticles at high doses. However, at lower doses of these particles, any of these 3 assays can be used. Prestoblue assay shows similar readings for the dose concentrations, however this should not be recommended to be used with these particles.

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18. HIT TO LEAD DEVELOPMENT FOR INHIBITORS OF S100P, A PROTEIN IMPLICATED IN PANCREATIC CANCER

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Background: Pancreatic cancer remains one of the top four most lethal forms of cancer after colorectal cancer, breast cancer, and lung cancer. It has a 20% survival rate in one year and less than 5% in five years with its current treatment, gemcitabine, not being effective.

S100P, a calcium-binding protein, is highly expressed in pancreatic cancer cells and is known to promote cell survival, migration and invasion. Studies have shown that cromolyn, an anti-allergic drug suppresses the cancer cell resistivity to chemotherapeutic treatment, reduces tumour growth, proliferation and metastasis of the cancer cells in animal models by inhibiting the normal interaction of S100P and the receptor for advanced glycation end product (RAGE).

When S100P interacts with RAGE, this triggers down-stream signals such as mitogen activated protein kinase (MAPK), nuclear factor light chain enhancer for activated B cells (NF_kB) and extracellular regulated kinase (ERK). Studies have shown that high levels of NF_kB are responsible for the drug resistance of the cancer cells. When S100P /RAGE interaction is inhibited and /or blocked, RAGE fails to trigger the aforementioned downstream signalling pathways resulting in reduced tumour growth, proliferation, metastasis and resistance to treatment of the cancer cells. Cromolyn is a weak inhibitor and has poor bioavailability.

Aim: The aim of our research is to design more effective inhibitors by computational modelling of S100P binding site, to enhance pancreatic cancer treatment. In this project, we intend to use S100P and computer simulation methods to study the interaction of S100P and cromolyn. This will enable us to identify the binding site of S100P and to generate a pharmacophore query which can be used for virtual screening of molecular data-base to identify inhibitors of S100P/RAGE interaction. Molecules identified from this interaction will be biologically screened for inhibition of S100P/RAGE interaction and for further studies in cancer cells.

19. DEVELOPMENT OF A NOVEL PATIENT-REPORTED OUTCOME MEASURE IN HAEMATOLOGICAL MALIGNANCY FOR USE IN ROUTINE CLINICAL PRACTICE: ITEM GENERATION

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Aims: The impact of haematological malignancies (HM) on patients' health-related quality of life (HRQoL) is still not well understood. The aim of this study was to identify HRQoL issues and symptoms in patients with haematological malignancy.

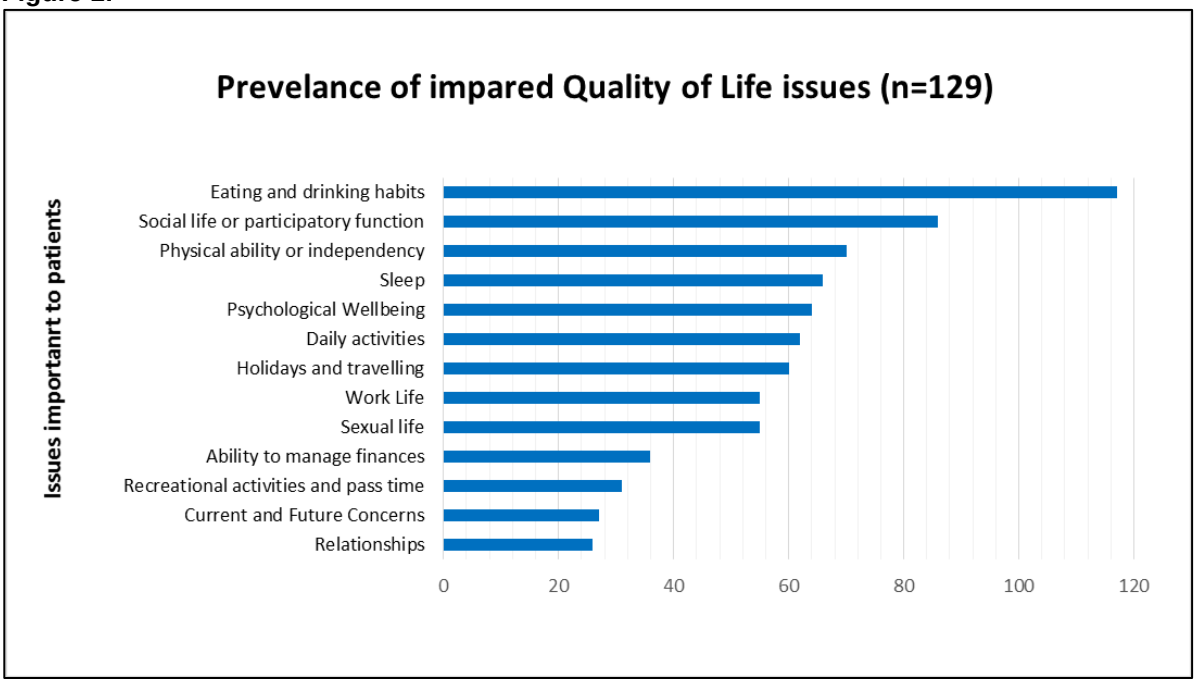
Methods: In a multicentre national observational study performed in the UK, adult patients with various HM , capable of reading English and able to give the written informed consent were recruited from five hospitals in England and Wales. This qualitative study employed semi-structured face-to-face interviews with open-ended questions related to the impact of haematological malignancy and its treatment on HRQoL and symptoms. All the interviews were audio recorded and transcribed verbatim and content analysis was carried out using the NVivo 11, qualitative analysis software. The themes and the sub-themes generated from the transcribed interviews were discussed during a 2-day "data definition" panel meeting by 2 haematologists, 1 patient research partner, 1 representative of a haematology patient organisation and 3 QoL research experts to select items for inclusion in the prototype instrument.

Results: 129 patients (Male=76; mean age = 61.1 years; SD=15.3; median age =64.9 years; age range =18-88 years) with mean duration of the HM of 3.60 years (SD=4.34; and range= 19 days-23 years) were recruited into the study. Diagnoses were Acute Myeloid Leukaemia (18), Acute Lymphoid Leukaemia (7), Chronic Myeloid Leukaemia (12), Chronic Lymphoid Leukaemia (11), Aggressive Non-Hodgkin's Lymphoma (16), Indolent Non-Hodgkin's Lymphoma (14), Hodgkin's Lymphoma (10), Multiple Myeloma (21), Myeloproliferative Neoplasm (10) and Myelodysplastic Syndrome (8). There were three hundred and eighty three item reported by the patients under different themes and subthemes. One hundred seventeen of these items were reported by more than 5% of the patients. One hundred and forty-nine items were selected by the data definition panel to be included in the prototype instrument. These items will be further re-grouped and refined using cognitive debriefing, content validity and factor analysis. The most prevalent and important QoL issues to HM patients (Figure 1) were: 'Eating and drinking habits (117); Impaired social life and participatory function (86); impaired physical ability or independency (70); disturbed sleep (66); impaired psychological well-being (64); and impaired daily activities (62); and impaired ability to go on holidays or travelling (60). With respect to disease related symptoms, 102 issues were identified, the most prevalent being 'tiredness (65), feeling unwell (28), breathlessness (24), lack of energy (21), and back pain (17). Out of 124 treatment related symptoms identified, the most prevalent were: 'tiredness (73); feeling sick (36); lack of energy (20); taste disturbance (20); and breathlessness (15).

Conclusion: The findings of the qualitative and item generation phase clearly indicate that HMs affect patients' QoL significantly. However, in the absence of a validated measure for use in routine clinical

practice, this is not captured in a systematic manner. Thus, this highlights the need for the development and validation of a new HM-specific PRO measure for use in such settings. Psychometric testing of the prototype instrument will be carried out to establish the measurement properties of the new HM-specific PRO measure.

Figure 1:



20. OPTIMISATION OF IMMORTALISED HUMAN KERATINOCYTE CELLS FOR THE DEVELOPMENT OF AN IN VITRO IRRITATION AND SENSITISATION MODEL

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Introduction: Robust safety assessment including the irritancy and sensitisation potential of new dermal therapies and cosmetics is required before they reach the market. Previously, the Draize test was used as a predictive model of chemical irritation and sensitisation using rabbits. Due to the new reforms and recommendations by the European regulation for Registration, Evaluation, Authorisation and restriction of chemicals (REACH), the drive to move away from animal based testing models has increased (Robinson *et al.,* 2002). Consequently, the European Union reference laboratory for alternatives to animal testing (EURL-ECVAM) has validated multiple human *in vitro* primary cell culture models as models for irritation and sensitisation testing (Fentem & Botham, 2002). Whilst these models are routinely used, there is considerable scope to improve the methodology and readouts for skin sensitisation and irritation assessment (Barratt *et al.,* 1998). The aim of this work is to explore the optimal set up of a novel human *in vitro* system to assess appropriate endpoints of dermal sensitisation and irritancy to topical treatments.

Methodology: Immortalised human keratinocyte cells (HaCaT) were used between passages 13-14 and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% v/v foetal bovine serum (FBS), 1% v/v penicillin/streptomycin and 2 mM L-glutamine. For experiments, cells were seeded on a 96-well plate at a density of 5×10^3 and 1×10^4 cells per 100 µl. Cell number and health were assessed every 24 h post seeding for 10 days. Cell number was determined via flow cytometry (Guava easyCyte HT sampling flow cytometer, Millipore). Cell health was assessed by the mitochondrial activity (MTT and MTS assays) and cell membrane integrity via the lactate dehydrogenase (LDH) assay.

Results and discussion: Exponential cell growth of HaCaT cells was observed between 1-4 days post seeding followed by a plateau phase for the remaining 6 days the cells were assessed. Both MTS and MTT assays produced comparable results for cellular mitochondrial activity. These results will be used alongside cellular functionality data to design an *in vitro* cell culture model which has been optimised for reporting sensitisation and irritancy.

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21. FABRICATION AND OPTIMISATION OF POLY (LACTIC-CO-GYLCOLIC ACID) (PLGA) MICROPARTICLES USING TAGUCHI ROBUST DESIGN

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Introduction: The human eye comprises of tissues with distinct features and characteristics, which restrict ocular drug from reaching the target site and tends to affect the therapeutic properties of drugs. The use of the conventional delivery system for treating ophthalmic diseases has several limitations, including low bioavailability and ocular effects. Microparticles have significant achievement in the pharmaceutical industries as one of the controlled drug delivery system for ophthalmic application. In this study, PLGA is chosen due to its biodegradable and biocompatible properties, control release, target delivery and therapeutic effects. The fabrication of PLGA microparticles is designed to provide controlled and sustained release, protect patients and other organs, and enhance the bioavailability of therapeutic drug. Comparative studies have showed that single emulsion evaporation method used for the preparation of microparticles is economical and robust (Bible et al., 2009). The particle size is important in determining the performance of the drug, duration of the drug release and mode of application (topical or injection). Our objective of the study was to use Taguchi robust design of experiment to develop and optimize various process parameters which affect particle size.

Methods: We identified ten parameters (concentration of Poly (vinyl alcohol) (PVA), molecular weight of PVA, concentration of PLGA, type of solvent (dichloromethane and ethyl acetate), concentration of PVA in hardening bath, steering speed, ratio of primary emulsion, vortex speed, duration of speed and evaporation) in the fabrication of 5-20*μm* PLGA microparticles using single emulsion method. Taguchi robust design was applied to study the influence of the ten parameters on particle size. Using design-expert software, L12 orthogonal array design was selected and used to explore two-level factor (lower and higher) for each of the parameters. The software presented an alias structure for L12 design, outlining the main effects and factor of interaction. Particle size and the units "*μm" was* entered as the response. Optical microscope and laser diffraction (Sympatec) will be used to analyze the particle size.

Results: The design-expert software generated a data of twelve experimental trials involving the ten parameters at lower and higher levels. The design required the experiments described in the L12 design data to be conducted and response entered for each trial. Using analysis of variance (ANOVA), the data will be analyzed to evaluate and identify the parameters which are statistically significant. A confirmation experiment is needed to verify the optimal design parameters.

Conclusion: The Taguchi robust method applied a minimal number of experimental trials, which is economical and time saving as compared to other factorial methods. The L12 design model is an ideal model to generate factorial experimental data for parameters less than eleven with two levels.

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22. 4,4'-DIMETHYLAMINOREX ('4,4'-DMAR'; SEROTONI) MISUSE; A WEB-BASED STUDY

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Introduction: 4,4′-DMAR (4,4'-dimethylaminorex; 'Serotoni') is a potent stimulant drug which has recently been associated with a number of fatalities in Europe. Over the last few years, online communities have emerged as important resources for disseminating levels of technical knowledge on novel psychoactive substances/NPS.

Objective: Analysing the information provided by the fora communities on 4,4'-DMAR use, additionally critical reviewing the available evidence-based literature on this topic.

Methods: Different website drug fora were identified. A critical review of the existing evidence-based literature was undertaken. Individuation and analysis of qualitative data from the identified website fora were performed.

Results: The combined search results identified six website fora from which a range of qualitative data on recurring themes was collected. These themes included: routes of administration and doses; desired effects; adverse effects; comparison with other drugs; association with other drugs; medications self-administered to reverse 4,4'-DMAR action; overall impression; provision of harm reduction advice.

Conclusions: Although being characterized by a number of methodological limitations, the social networks' web monitoring approach (netnography) may be helpful to better understand some of the clinical and psychopharmacological issues pertaining to a range of NPS, including 4,4'-DMAR, for which only extremely little, if any, scientific knowledge is available.

23. ANTI-INFLAMMATORY ACTIVITY OF NOVEL TRANS-STILBENE SULFONAMIDE ANALOGUES AS POTENTIAL NOVEL THERAPEUTIC AGENTS FOR LUNG DISEASE

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Introduction: Chronic obstructive pulmonary disease (COPD) is a progressive disease of the airways, leading to chronic inflammation of the lung and is the fifth major cause of mortality in the UK [1]. Causative agents such as particles from tobacco smoke, biomass exposure and pollution cause airway inflammation, resulting in the release of reactive oxygen species in the airways [2]. Oxidative stress is an important factor in the pathogenesis of COPD and as such offers a perspective target for treatment of the disease [3]. Natural peanut stilbenes such as resveratrol (trans-3,5,4-trihydroxystilbene) and synthetic stilbene analogues have been shown to exhibit a number of clinically useful biological properties, including an anti-inflammatory mode of action [4]. The main issues preventing resveratrol and other stilbene analogues from being marketed drugs are their low oral bioavailability as a result of poor aqueous solubility and rapid liver metabolism [5, 6]. However, these properties may not hinder delivery via the inhaled route for the management of local airway inflammation and oxidative stress. Current treatment options for COPD are limited and do not prevent disease progression. Hence, there is an urgent need for alternative novel therapeutic approaches. Reactive oxygen species play a key role in oxidative stress which is associated with COPD pathogenesis. Hence, oxidative stress is a potential target for new therapies for lung inflammatory disorders. In this study, characterisation of a library of stilbene sulphonamide compounds for their impact on cell health and inflammation was carried out *in vitro*.

Methods: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess novel stilbene compounds for anti-oxidant free radical scavenging activity in accordance with Trotta et al. [7]. Stilbene compound solutions were prepared in ethanol (0.1 µM-100 µM) and combined with 60 µM ethanolic DPPH solution. Resveratrol (100 µM) and ascorbic acid (10 µM) were used as positive controls. Samples were incubated at room temperature in the dark for 30 min and absorbance was read at 540 nm. The (2',7' – dichlorofluorescein diacetate (DCFDA) cellular reactive oxygen species assay kit was used to measure reactive oxygen species within the cell following induction of oxidative stress. The protocol was conducted per manufacturer's guidelines (Abcam, Cambridge, UK).

Results and Discussion: There was no significant impact observed on cell health at concentrations below 50 µM, indicating their suitability as safe inhaled therapeutic agents. Some of the analogues have shown promising anti-oxidant potential *in vitro*, displaying comparable activity as resveratrol at 10 µM concentrations. In particular compound 1A was observed to possess strong anti-oxidant activity at 1 µM.

Conclusions: Preliminary SAR evaluation of stilbene compounds has shown that methoxylation of resveratrol analogues enhances anti-oxidant potential. Further structure activity relationship studies are required to help further elucidate the mechanism of action of these compounds. These preliminary investigations have indicated low toxicity and moderate activity against oxidative stress *in vitro*. The library

of trans-stilbene sulphonamide analogues warrant further investigation to assess their potential use for airway diseases such as COPD and asthma.

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24. ANALYSIS OF LEGAL HIGHS SUBSTANCES USING HANDHELD RAMAN SPECTROSCOPY

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Introduction: The rapid rise of new psychoactive substances (NPS) has been highly noticed in the last decade. This has been mainly caused by illegal chemical practices in clandestine laboratories by criminal organisations which have accentuated their availability through the illicit market. Few analytical techniques have been of use in the identification and detection of these fast-growing substances. However, due to the restrictions of sample preparations posed by most of them, our attention was turned to Raman spectroscopy particularly the handheld Raman instrument. This technique was helpful in the analysis of NPS mixtures and is particularly advantageous due to minimum sample preparation needed. However, the use of cutting agents and adulterants have been found to impact on the detection of NPS substances. Therefore, the purpose of the research focussed on the relation between the concentration of an NPS and its detection within a mixture.

Methods: Samples were made based on 5 components which were the NPS in this instance 5F-PB-22 (a synthetic cannabinoid) and 4 excipients which were caffeine, benzocaine, creatine and sodium glutamate. However, given the high numbers of possible combinations, a design of experiment using a simple algorithm was used resulting in 26 samples where the concentration of each component alters in accordance to cover the broad variety. NPS samples were to challenge the handheld Raman instrument and determine what were the limits of detection (LoDs) for NPS of interest and the complexity involved in its detection in regards with other components.

Results: Matching results of 5F-PB-22

The data collected from the analysis of 5F-PB-22 highlighted that in case of high concentration, the NPS get identified as a top hit. On the other hand, some components present in considerable concentration were not always detected in some vials. Sodium glutamate appeared to be mostly undetected unless present in majority unlike benzocaine always detected unless when absent.

Discussion: This was due to the spectral properties of each adulterant. In fact, benzocaine is a very good light scatterer due to its structure and is mostly detected by Raman spectroscopy even when present at low concentrations in contrary to Na glutamate. This could therefore pose a problem in detecting and identifying other components. This interpretation therefore suggests that the LoDs of each NPS can be significantly impacted by not only the concentration of present excipients within the mixture but also by their spectral behaviour regarding to Raman.

25. DETERIORATION DUE TO MEDICINES RELATED PROBLEMS IN OLDER ADULTS IN PRIMARY CARE – FEASIBILITY OF SYSTEMATIC REVIEW STRATEGY

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Introduction: At 75 years and over, the ageing process becomes clinically significant in terms of drug handling. This may result in medicines related deterioration in health. The definition of deterioration in primary care setting used in this review is as follows: hospitalisation; increased use of health care services; falls; delirium; pressure ulcers or death. Though, a lot of work has been undertaken on medicines use in older people none to the knowledge of the reviewers focused on deterioration related to medicines in primary care. Hence, this systematic review aims to identify MRPs and associated risk factors that may contribute to deterioration in health of older people in primary care with the objectives set to: (a) assess published studies addressing medicines related problems that cause deterioration and (b) identify interventions used and the success of these to address deterioration due to medicines. The strategy adopted to undertake the systematic review is reported in this paper

Methods: A strategy was established for study identification, quality assessment, synthesis and analysis. Relevant literature published between 2001 to date was accessed through a systematic search of relevant databases using relevant search terms: adverse drug event, adverse drug reaction, medication error, pharmaceutical care issues, pharmaceutical services, primary health care, patient discharge, continuity of patient care, doctors office, ambulatory care, surgery, accident and emergency, pressure ulcers, delirium, fall, hospitalisation, geriatric syndrome, death.

Results: Potential relevant articles identified were 75. Three of these articles were piloted to test feasibility of the strategy**;** MRP-related deterioration: Two of the studies reported hospitalisation (Hofer-Dueckelmann, et al., 2011); (Laattikainen, et al., 2016) one of the studies reported vertigo, falls, fractures attributable to MRPs (Laattikainen, et al., 2016). Severity, causality and preventability of MRP-related deterioration in older people: Two of the studies reported causality one measured by Naranjo scale (Hofer-Dueckelmann, et al., 2011) while one used both the Naranjo scale and expert opinions to decide on causality (Laattikainen, et al., 2016). The degree of causality in the two studies ranged from 13% to 74%. One of the studies measured neither severity nor avoidability (Laattikainen, et al., 2016). One of the studies measured severity and avoidability but did not report on out outcome (Hofer-Dueckelmann, et al., 2011). Reported MRPs, predictors and risk factors: Two of the studies reported on Adverse drug reactions (Hofer-Dueckelmann, et al., 2011) (Laattikainen, et al., 2016), while one study reported on adverse drug event and drug-drug interaction (Laattikainen, et al., 2016); predictors were reported by one study as age and number of medicines (Hofer-Dueckelmann, et al., 2011) while one study reported poly pharmacy as a risk factor($p=0.01$) with age being a predictor to polypharmacy ($p=0.001$) (Laattikainen, et al., 2016). Drugs most commonly implicated: Two studies reported CVS drugs as those involved in deterioration. The other

classes reported by single studies were CNS, NSAID and anti-neoplastic. Interventions to stop deterioration: The study on pharmacist intervention targeting polypharmacy did not yield improved health outcomes (Sellors, et al., 2003).

Conclusion: Search terms for MRPs may need to include inappropriate medicines for additional sensitivity.

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26. EXPLORING THE ROLE OF EPIGENETIC. *IN-SILICO* INVESTIGATIONS OF ANTIMICROBIAL CATIONIC PEPTIDE CONFORMATIONS IN THE PRESENCE OF WATER AND MICELLES

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Introduction: The surge of multidrug resistant microorganisms and the lack of new antibiotics entering the market is posing a serious risk to public health and is being recognized as one of the major emergencies in modern medicine (Liu et al., 2016). Cationic antimicrobial peptides can be found in many natural sources and represent a very promising class of molecules to be investigated for the development of new antibiotics (Domalaon, G Zhanel, & Schweizer, 2016). It has been suggested that these molecules bind and consequently disrupt the cellular membrane of both Gram negative and positive bacteria. The aim of our work is to explore a protocol for the *in silico* evaluation of their mechanism of action that can be used in high throughput screening for antimicrobial activity of these peptides and related peptido-mimetics.

Method: Simulated annealing (SA) and molecular dynamics (MD) simulations were performed on three representative antimicrobial cationic peptides in the presence of randomly distributed water and either octanol or dodecylphosphocholine (DPC) molecules. The simulations were carried out using Desmond software and OPLS-2005 force filed using Maestro as graphic user interface.

Results: It was observed that the hydrophobic molecules were forming micelle like higher order structures at the end of SA simulations, allowing the study of peptide conformations in the presence of membrane like environment. All three peptides were partitioning at the interface between hydrophobic surface and the aqueous environment. Interestingly, some of peptides would form conformations that closely resemble their experimentally determined structures in presence of micelles using NMR.

Discussion and conclusions: The simulated results are in general agreement with the suggested mechanism of action of these peptides and the NMR data obtained on the crystal structures of these peptides in presence of octanol or DPC micelles reported in literature. This provides a good basis for a development of a novel protocol for *in silico* prediction of peptide ability to exert antimicrobial activity.

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27. THE USE OF AN *IN VITRO* ARTIFICIAL "THROAT" MODEL TO EVALUATE SAFE SWALLOWING OF COMMERCIAL JELLIES AND THICKENED FLUIDS

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Introduction: Modifying the consistency of food or liquid is a common intervention for patients with dysphagia. Thickened fluids are used in practice to deliver crushed tablets or capsule contents for patients with swallowing difficulties. Although thickened fluids reduce the risk of penetration-aspiration, patient acceptability is low. Other foods such as jellies are used as swallowing aids for medicines-taking, negating the need of water to swallow tablets and capsules. However, there is no information available to suggest other swallowing aids such as jellies are safe to swallow by dysphagia patients. The aim of this project is to compare the safety features of commercial jellies and thickened fluids using an *in* vitro artificial "throat" model.

Methods: An *in vitro* mechanical 'throat' model was used to compare the flow of three commercial jellies to three commercial dysphagia thickeners. Commercial thickeners were thickened to three stages (nectar, honey, and pudding thick) as recommended for patients with different severity of dysphagia. Commercial jellies were manually cut to particle sizes of approximately 4mm to account for chewing. Movie photography captured images of the flow of each sample in the model, and the time taken for 'boluses' to reach the epiglottis and oesophagus was calculated. Commercial jellies and thickened fluids were characterised by obtaining rheological data on steady state flow, linear viscoelasticity, and frequency sweeps. Texture parameters such as cohesiveness and surface adhesion were obtained using a back-extrusion rig on a TA.XT Plus Texture analyser.

Results and Discussion: All of the products showed the same characteristic rheological features with shear-thinning flow and G' dominance indicating gel-like structure. Stages one and two thickened fluids showed fast and turbulent flow in the *in vitro* "throat" model with no cohesion of the bolus observed. An increase in transit time and bolus cohesion were observed for stage three thickened fluid and jellies. The time taken for "boluses" to reach oesophagus correlates with cohesiveness of the material.

Conclusions: Commercial jellies displayed similar safe swallowing behaviour to dysphagia thickeners at stage three thickening, showing promise as a swallowing aid for patients with dysphagia.

28. INHIBITION IN MODULATING HELPER (CD4+) AND CYTOTOXIC (CD8+) T CELL FUNCTION

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Introduction: The term, 'epigenetics' refers to heritable changes in phenotype that are independent of changes in the underlying DNA sequence (Arrowsmith, 2012). Epigenetics allows for the development of cell lineages that are functionally distinct but genetically identical, since the phenotype of a cell is determined to a large extent by the pattern in which its thousands of genes are expressed. More recently, the term epigenetics has encompassed all changes in gene expression that result from changes at the level of chromatin, the complex structure in which DNA is packaged within proteins in the cell nucleus, regardless of the duration of these effects. CD4+ and CD8+ T cells play an important role in the orchestration and adaptation of the host immune response, but can also contribute to autoimmunity and inflammation. In an *in-vivo* setting, dependent upon the context of the stimulus and cytokine milieu present during activation and expansion, T cells undergo myriad alterations which regulate gene expression of cytokine and other gene loci, much of which is determined epigenetically. As a result, inhibition of these epigenetic processes presents a promising new arena for small molecule intervention to limit the production of pro-inflammatory and cytotoxic mediators from immune cells, including T cells.

Methods: In order to investigate the effect of epigenetic modulation upon the activation, proliferation and effector functions of T cells *in-vitro*, the total CD4+ or CD8+ T cell compartment was isolated from peripheral human blood using specific antibodies and magnetic beads. Characterisation of cell populations was carried out pre and post stimulation through the T cell receptor in the presence of epigenetic inhibitor compound or control. Flow cytometric analysis was used for determination of cell activation, proliferative capacity and cellular viability, alongside immunoassays performed on cell supernatants in order to assess the ability of the cells to produce effector molecules such as IFNγ and granzyme B. In addition, mRNA production was assessed at 24 hours post cellular activation by RT-qPCR.

Results: Epigenetic inhibitors potently modulate the expression of effector molecule production from both CD4+ and CD8+ T cells, with additional inhibition of proliferation, and modest effects on cellular viability.

Conclusions: Targeting epigenetic proteins results in profound anti-inflammatory activity, and as such may have therapeutic potential in immune-mediated inflammatory disorders**.**

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29. THE DEVELOPMENT OF A 3D, HUMAN OCULAR EYE MODEL FOR REGENERATIVE MEDICINE AND OCULAR DELIVERY

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Introduction: Limitations associated with animal models using rabbit, chicken and bovine eyes have encouraged the development of more accurate *in vitro* cell-based models that aim to replicate the growth conditions of native tissue. These models are used to investigate cell behaviour and to provide an alternative approach for drug toxicity testing (1). However, there is still a need for *in vitro* models that are not only physiologically relevant but also take into account the native organs' geometry and growth conditions. In this study, we aim to develop a 3D *in vitro* ocular model which mimics both the cellular microenvironment and the physico-mechanical properties of the eye—thereby offering a potential screening tool for drug development studies.

Methods: Human retinal (ARPE-19) and corneal epithelial cells (HCE-2) cells were cultured on flat, curved and v-shaped well plates/scaffolds. Cells were characterised for morphology, activity/viability, phenotype and functionality using a combination of microscopy, biochemical assays (i.e. MTS, LDH release, Guava ViaCount), and flow cytometry. Mechanical characterisation of vitreous humours from a number of mammalian candidates (bovine, porcine and sheep) and biomimetic substitutes (i.e. sodium hyaluronate, agarose, gelatin) were examined using rheometry, texture profile analysis, and scanning electron microscopy. In addition, *in vitro* diffusion studies were carried out using a novel Franz cell set up and steady-state flux through optimal vitreous humour substitutes were measured and compared to mammalian vitreous in order to validate these substitutes.

Results and discussion: Results demonstrate that cell activity and viability were adversely affected by extreme surface geometry (i.e. v-shaped scaffolds). In contrast, no significant impact on cell functionality or phenotype occurred during culture between flat and curved environments. A number of vitreous humour substitutes offer promising characteristics for an optimal biomimetic human vitreous replacement by demonstrating comparable properties (e.g. viscosity).

Conclusions: It can be concluded that ocular cells' characteristics were retained upon culturing on growth scaffolds mimicking native organ's geometry. In addition, an optimal replacement for human vitreous humour with comparable physio-chemical characteristics was established. These results are promising in developing a 3D, *in vitro*, ophthalmic model consisting of retinal cells, corneal cells and vitreous humour that closely represents in vivo growth microenvironment. This model can be exploited as a more biologically-relevant model for toxicity testing and drug delivery applications.

Acknowledgement: The authors would like to thank the University of Hertfordshire for funding and the Institute of Ophthalmology/Moorfields Biobank, UCL, for providing the human vitreous used in this study.

Reference:

1. Shafaie S, Hutter V, Cook MT, Brown MB, Chau DYS. 2016. *In Vitro* Cell Models for Ophthalmic Drug Development Applications. BioRes. Open Access, 5, 94-108

30. ASSESSMENT OF COMMUNITY PHARMACISTS' KNOWLEDGE, ATTITUDE AND PRACTICE REGARDING NON-PRESCRIPTION ANTIMICROBIAL USE AND RESISTANCE IN THAILAND- A QUESTIONNAIRE DEVELOPMENT

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Introduction: Antimicrobial resistance (AMR) is a global threat issue and increasing for decades. Community pharmacists are expected to provide appropriate medicines and information to their patients. However, several pieces of research indicate that community pharmacists were inadequate knowledge in antimicrobial resistance and inappropriate dispensing (Apisarnthanarak et.al, 2008). This study aims to assess knowledge, attitude and practice of community pharmacist in Thailand regarding non-prescription antimicrobials use and resistance. A questionnaire development is a part of the project. It was developed by literature reviews and base on Knowledge, Attitude and Practice (KAP) model for assessment antimicrobial provision in Thai community pharmacists.

Methods: Literature in antimicrobial resistance, antimicrobial use and Knowledge-Attitude-Practice theory were reviewed. The questionnaire was constructed by KAP model.

Results: A questionnaire contains 38 questions. It divided into four sections to assess pharmacists 'knowledge, attitude and practice regarding non-prescription antimicrobial use and resistance. **Demographics section** asks 5 questions to describe respondents' characteristic. It asks personal background, educations, experiences, workplace location and type of pharmacy. **Knowledge section** evaluates understanding of pharmacist in antimicrobial use and resistance. It was split into 2 topics as knowledge of antimicrobial use and knowledge of resistance (WHO, 2015). Knowledge of antimicrobial resistance topic asks 5 questions in a principle of the resistance, antimicrobial consumption and antimicrobial stewardship. Knowledge of antimicrobial use topic has 5 questions includes pharmacology, patient safety and rational use of antibiotics follow by a guideline in Thailand (Apisarnthanarak et al., 2008; Chongtrakul, 2011; Chuenchom et al., 2016). **Attitude section** examines agreement of pharmacist in antimicrobial use and resistance. It provides 10 statements in the topics for respondents give their agreement by the level of agreement scales as; Strongly disagree, Disagree, Neither agree nor disagree, Agree and Strongly agree (Roque et al., 2014). **Practice section** investigates current pharmacists' actions in pharmacy according to their knowledge and attitude for providing antimicrobials. The actions was developed by literature in role of pharmacists in antimicrobial use (WHO, 2014). The participant will self-evaluate their 10 actions by frequency scales by frequency scales as Never, Rarely, Sometimes, Often and Always for responding. Also, 3 open-end questionnaires ask respondents about common infectious diseases, and antimicrobials use in pharmacy and reason to provide non-prescription antimicrobials for patients.

Conclusions: A 38-item self-administration questionnaire was developed following a literature review in antimicrobial use and resistance issue. It was constructed based on KAP theory to assess community pharmacists' knowledge, attitude and practice in Thailand.

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31. DEVELOPMENT AND EVALUATION OF ADME MODELS USING PROPRIETARY AND LITERATURE DATA

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Introduction: ADME (Absorption Distribution Metabolism Elimination) properties are important factors for the fate of drugs through the drug discovery pipeline. It is significant to predict the ADME of a drug from the early stages and two factors can help to do that: 1. advances in the development of QSAR predictive models and 2. literature data, often collected in large databases such as ChEMBL (Gaulton et al., 2012; Bento et al., 2014). Pharmaceutical companies build ADME QSAR models using proprietary data and the inclusion of literature data might be a valuable source for the development of predictive models. The current study is investigating whether merging literature and proprietary data can improve the predictive activity of proprietary models in Evotec. This work is divided in two parts. The first part aims at evaluating existing Evotec permeability model and its applicability domain (AD) by estimating the confidence in the predictions based on the distance of the literature compounds from the model. The second part involves the development of models with proprietary and literature data and their comparison with proprietary models.

Methods: Different distance to model metrics were used in the descriptor space for the estimation of the AD: Mahalanobis distance (MD), Leverage and k-Nearest Neighbour (k-NN) with Euclidean distance (ED) and Manhattan distance (ManD). In the subsequent part, two new permeability models were built. Partial Least Squares (PLS), Random Forest (RF) and Support Vector Regression (SVR) with radial basis function (rbf) have been used to develop the QSAR models. Temporal test sets, (i.e. including experimental measurements published after the model were build) of public and proprietary data were used to assess the models.

Results: The results showed that despite the proprietary (Evotec) models could predict with good accuracy newly synthesized proprietary compounds; they were not as accurate when used to predict the permeability of public compounds. The results of the distance to model metrics indicated that there is weak relationship between the Root Mean Square Error (RMSE) and the distance of literature compounds from the Evotec proprietary compounds, which were used as the training set of the existing permeability model. Different thresholds applied to each distance to model metric to identify which compounds fall within the AD of the existing permeability model. The results obtained from each method were different but there were many compounds both within and outside the AD. Finally, the comparison of the models showed that the merging of literature with proprietary data slightly improves the prediction of proprietary temporal compounds, but it can significantly improve the prediction of temporal compounds extracted from literature. Similar results obtained with the three different algorithms but RF and SVR performed better compared to PLS.

Conclusions: In conclusion, none of the distance to model metrics proved to be superior to another and it is more accurate to use more than one of these methods to investigate the AD of a model. Compounds extracted from literature were within and outside the AD. This is an indication that their inclusion might be

beneficial because they can possibly introduce chemical diversity to the existing proprietary models. Finally, the new models, into which the literature compound were incorporated, are able to better predict new temporal compounds, extracted from literature, compared to proprietary models.

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PSYCHOLOGY

32. TAKING THE WEIGHT OFF: EVALUATING A TIER 3 WEIGHT LOSS CENTRE BASED ON WEIGHT MEASUREMENTS AND WELLBEING SCORES

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Introduction: In the UK 26% of all adults in England are obese (BMI ≥ 30). Latest estimates suggest over £10 billion, approximately 10% of the UK National Health Service budget is spent on obesity related disorders (NIHR, 2016). The World Health Organization (WHO) considers Obesity as such a big issue that it was formally recognised as a global epidemic in 1997 (Caballero, 2007). National Director of Health and Wellbeing for Public Health England, Professor Fenton, suggests Obesity will overwhelm the NHS within the next 10-20 years if not tackled now (Fenton, 2016). Health care professionals are the first to offer help and advice to patients with weight issues. The NHS advice recommendations for the treatment of obesity includes four main suggestions; diet, exercise, medication (Orlistat) and in extreme cases bariatric surgery (NHS England, 2014a, 2014b, 2015). The NHS has an obesity care pathway for England (Barth, 2015). The Rotherham Institute for Obesity (RIO) is one of a handful of tier 3 weight loss services across the UK. RIO offers a multi discipline approach tackling obesity. RIO consists of eleven members of staff, these health professionals. Including a GP, exercise therapists, talking therapists, obesity specialist nurses and health care assistants play a key role in educating, encouraging, and supporting patients' weight loss.

Methods: Between 2009 -2015 each patient's measurements had been taken at each appointment. RIO recorded 7,353 adult patient referrals; 4,680 of these patients were referred once, with 2,673 patients reappearing at RIO for a further referral. Patients are split in to two groups, first referrals Group 1 (4,680 patients), patients with multiple referrals Group 2 (2,673 patients). The analysis will be undertaken using parametric tests of the mean scores. However, as RIO works with patients who are at the higher end of overweight, this potentially skews the data. During the analysis, the distribution will be tested further and non-parametric tests of the medians carried out.

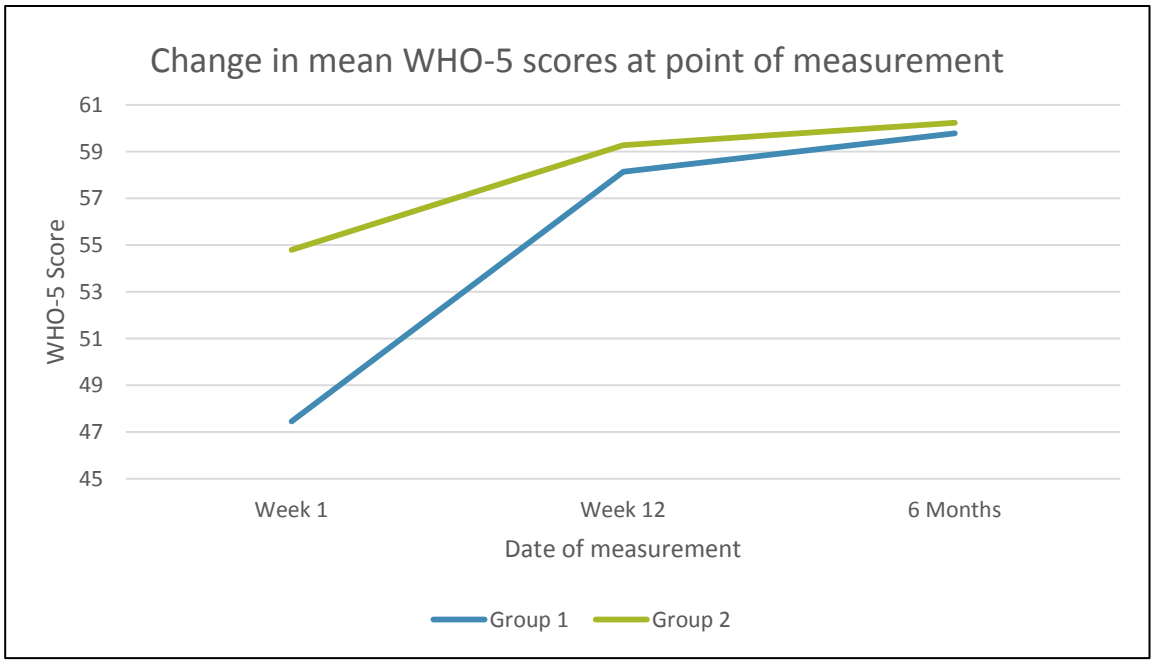
Results: Analysis will test the differences between measurements to compare the two groups on, weight loss, change in waist circumference and change in WHO5 scores. Patients' lost weight and reduced their waist circumference with RIO are shown in Table 1. During their time with RIO patients' mean wellbeing score increased at each point of measurement for both Group 1 and Group 2 (Figure 1).

Table 1: Body measurements for RIO patients' percentage weight loss and percentage reduction in waist circumference by group

Weight Loss (kg)		**Week 1-4**	**Week 1-8**	**Week 1-12**	**Week 1-6**
Group 1	Mean	1.46	2.38	2.32	11.56
	Median	1.29	2.18	1.70	5.97
Group 2	Mean	.75	1.24	1.28	11.38
	Median	.63	1.01	.59	4.33
Waist Circumference (cm)		**Week 1-4**	**Week 1-8**	**Week 1-12**	**Week 1-6**
Group 1	Mean	.77	2.60	3.39	5.38
	Median	1.12	2.48	3.33	5.38
Group 2	Mean	1.10	1.92	1.43	3.33
	Median	.68	1.30	1.61	2.91

Median percentage change week 1-4, 1-8, 1-12, 1-6 months are the same across group categories (Mann-Whitney U-Test, $p < .000$)

Figure 1: Patients' WHO-5 mean change in wellbeing scores at points of measurement during their time with RIO



Discussion and Conclusions: Patients in both Group 1 and Group 2 lost weight with RIO at a similar rate. This shows that weight loss can be successfully achieved for both single and multiple referrals at RIO. Success was also seen in the reduced patients' waist circumference size; Group 1 and Group 2 both showed a reduction in size. Patients achieved a bigger reduction during their first referral at RIO, with this reduction occurring at a smaller rate for patients that return for multiple referrals. The WHO-5 scores for patients have been calculated using the formula set by the WHO. This showed that patients' wellbeing

scores increased during their time with RIO. These findings show that the methods RIO use work, allowing patients to both lose weight and improve their wellbeing whilst at RIO.

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33. SEE IT, FEEL IT, OWN IT: HOW GALVANIC VESTIBULAR STIMULATION ENHANCES BODY OWNERSHIP DURING THE RUBBER HAND ILLUSION

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Introduction: Body ownership refers to the sense that one's body belongs to oneself. This facet of bodily self-consciousness relies on the integration of sensory signals from several different sources, including traditionally exteroceptive information (e.g. vision) and interoceptive information (e.g. proprioception and affective touch). The vestibular system plays a key role in managing the balance between various sensory systems and their contribution to body ownership. However, it is still unclear how exactly these different sources of information contribute to body ownership. The aim of the current study was to examine how the vestibular system affects the relative influence of these different sensory modalities to body ownership.

Methods: We used galvanic vestibular stimulation (GVS; Ferrè, Berlot, & Haggard, 2015; Lopez, Lenggenhager, & Blanke, 2010) to stimulate the vestibular system of healthy participants during an experimentally induced body ownership illusion (i.e. the rubber hand illusion; RHI; Botvinick & Cohen, 1998). Participants took part in a RHI procedure, during which they observed a realistic rubber hand being stroked in or out of synchrony with their own unseen hand. This synchronous (but not asynchronous) stroking typically increases ownership of the rubber hand (as indexed via questionnaires and the perceived drift in location of the real hand towards the rubber hand; i.e. proprioceptive drift). We compared rubber hand ownership in 26 healthy participants during conditions of: (1) vestibular stimulation resulting in activation of the right- vs. left-hemisphere of the brain vs. sham stimulation, (2) synchronous vs. asynchronous stroking of the real and rubber hand, and (3) affective (slow) vs. neutral (fast) velocity stroking. This resulted in a 3 (GVS stimulation) x 2 (stroking synchrony) x 2 (stroking velocity) within-subjects design. In addition, to examine the effect of vestibular stimulation during vision alone (i.e. without tactile stimulation) on body ownership (referred to as 'visual capture') we performed the same three types of stimulation while participants simply looked at the rubber hand.

Results: We found that right-hemisphere stimulation (as compared to left hemisphere or sham stimulation) significantly increased visual capture (i.e. there was a significant increase in proprioceptive drift) during the vision only conditions. In the touch conditions, right-hemisphere stimulation during synchronous stroking also increased proprioceptive drift compared to the stimulation of the same areas in left hemisphere. Finally, right-hemisphere stimulation resulted in affective (i.e. slow, gentle) touch leading to a significant increase in proprioceptive drift compared to neutral (i.e. fast) touch during synchronous stroking conditions.

Conclusions: Our study revealed that right-hemisphere vestibular stimulation influences the balance of the different sensory modalities contributing to the sense of body ownership. Specifically, right-hemisphere vestibular activation enhances visual information during the rubber hand illusion, thereby reducing the

influence of proprioceptive signals. In addition, using slow, affective touch during right-hemisphere activation further enhanced the dominance of visual information over proprioceptive signals. Our findings highlight the importance of the vestibular system in managing the balance of sensory information that contribute to body ownership, and suggest an important role of affective information (i.e. slow gentle touch) in the way we perceive our body.

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34. COULD NEUROCOGNITIVE MARKERS BE USED TO GUIDE AND OPTIMISE TREATMENT IN OBSESSIVE-COMPULSIVE DISORDER?

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Introduction:

Obsessive Compulsive Disorder (OCD) is a common and highly functionally disabling disorder affecting 1-2% of adults in the United Kingdom (Torres et al., 2000, Pinto et al., 2006). It is characterised by intrusive and unwanted thoughts, urges or impulses and repetitive, compulsive behaviours or mental rituals that are associated with significant distress and anxiety. Current treatment guidelines recommend either Selective Serotonin Reuptake Inhibitors (SSRIs) or Cognitive Behavioural Therapy (CBT) either individually or in combination. However, only approximately 50% respond and, we have no reliable means of predicting which patients will respond to which treatment. There is some evidence that neurocognitive function is impaired in OCD (Dittrich et al., 2013; Fineberg et al., 2014; Bandelow et al., 2016). It has been hypothesised that the quality or degree of cognitive impairment found in an individual could be used to predict response to treatment. These measures include;

- Motor impulsivity
- Attentional set-shifting
- Affective bias
- Decision-making

A review of the published OCD literature reveals several studies indicating a relationship between altered response to treatment and neurocognitive function with a differential effect on drug and psychological treatment. For example, D'Alcante et al. (2012) found that greater cognitive flexibility predicted a better response to CBT for treatment-naïve OCD patients. Fontenelle et al. (2001) demonstrated impaired set-shifting abilities predicted a better treatment response to SSRIs.

Aims: The aims of this study are to determine whether changes in selected neurocognitive domains known to be associated with OCD predict treatment response to SSRI, CBT or combination therapy, which could in future be utilised to guide tailored therapy.

Method: As part of the ongoing Optimal Treatment for OCD (OTO) study, 60 patients with Obsessive-Compulsive Disorder were randomised to one of three treatment arms: SSRI monotherapy (Sertraline) for 52 weeks, 16 hours of CBT or combination therapy for a total of 52 weeks. Neurocognitive testing was performed at baseline, 16 weeks and 52 weeks to assess the four cognitive domains outlined above. The Yale-Brown Obsessive Compulsive Scale (YBOCS) score was also assessed at the same time-points. When the data collection is completed, an appropriate method of statistical analysis will be applied to explore relationships between these neurocognitive characteristics and change in symptom severity (as measured by the change in YBOCS).

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SPORT, HEALTH & EXERCISE

35. THE EFFECTS OF GENDERS AND LOAD POSITION ON LOWER EXTREMITY BIOMECHANICS DURING JUMP LANDINGS

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Introduction: Both gender and additional load have been shown to affect landing mechanics. Females are at an increased risk of sustaining an anterior cruciate ligament injury and patellofemoral pain syndrome, whereas males are at a greater risk of developing patellar tendinopathy. There is a paucity of literature examining the effects of load position or the comparison between genders during jump landings with additional load. The purpose of this investigation was to examine the effects of different positions of loading on lower extremity biomechanics during a jump-landing task. An additional purpose was to compare lower extremity biomechanics between genders during loaded and unloaded jump landings.

Method: Twelve resistance trained males (age 21.1 ± 1.4 years, body mass 76.2 ± 10.3 kg, height 1.77 ± 0.08 m) and 12 resistance trained females (n = 12, age 20.3 ± 1.4 years, body mass 64.4 ± 7.2 kg, height 1.70 ± 0.03 m) were recruited. Three-dimensional lower-limb-joint kinematics and kinetics were measured during 5 bilateral maximal jumps were performed in a randomised order in each of four conditions: unloaded (UL), holding dumbbells (DB), wearing a weighted vest (WV), and with a barbell placed across the shoulders (BB). All loaded conditions were performed with 10% of body weight. A two-way analysis of variance (type of load * gender) was performed on kinetic and kinematic variables. Cohen's d effect sizes were calculated for differences between load types.

Results: Significant differences were shown between genders with the male group exhibiting increased jump height, lower time to peak knee flexion, smaller sagittal plane excursion (ROM), and smaller hip adduction angles at ground contact (IC) and smaller peak hip adduction angles when compared to females. Males were also shown to have significantly greater peak vertical ground reaction force (vGRF), significantly smaller time to peak vGRF, and significantly greater peak knee and hip extension moments. There was no significant interaction between load and gender in all variables measured. All loaded jumps resulted in a significant increase in the time to reach peak knee flexion. Significant decreases were observed in peak hip adduction moment and peak knee valgus moment in the male BB condition and female BB condition respectively when compared to UL jump landings. There was a significant decrease exhibited in vGRF in both genders in the BB condition when compared to the UL condition.

Conclusions: Both gender and additional loading alter landing biomechanics in maximal vertical jump landings. Furthermore, the position in which the load is placed changes landing biomechanics. Changes in landing strategy with additional load were found to be similar in both genders.

36. WRIST-WORN ACCELEROMETER MEASURES OF MOVEMENT BY PEOPLE WITH PARKINSON'S DURING AND FOLLOWING DANCE CLASSES AT THE UNIVERSITY OF HERTFORDSHIRE.

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Introduction: People with Parkinson's have anecdotally reported that the physical and psychological benefits of attending a dance class are maintained for the hours immediately following the class; however these may wear off over the subsequent days. The use of a wrist-worn accelerometer can provide an objective, yet non-invasive, way of quantifying the amount of movement made by people during and after such activities (Eslinger, Rowlands, Hurst, Catt, Murray & Eston, 2011). To date, research has focused on using the devices to classify specific movements, such as tremor, over a short period of time. The purpose of the current study is twofold: firstly, to measure the amount of movement made by people with Parkinson's compared with age-matched controls and younger individuals during a dance class; secondly, to measure the amount of movement by people with Parkinson's over the week following a dance class compared with a week when they do not attend a dance class.

Methods: People with Parkinson's and age-matched controls who regularly attend a dance class at the University of Hertfordshire were asked to wear an accelerometer on their wrist as they took part in a dance class and then on a separate occasion for seven days following the dance class. They were not asked to do anything specific other than continue their daily routine as normal and to keep a brief diary of their activity over the seven days. Participants then wore the accelerometer during a week when they did not attend a dance class to allow a comparison between the amount of movement made on a dance and non-dance week. In addition, data collection is currently underway over a week for people with Parkinson's who do not attend dance classes.

Results: Feedback from the participants has been positive about wearing the accelerometers, indicating the feasibility of using this device to measure activity over a sustained period of time. Accelerometer measures revealed that during the dance class people with Parkinson's (N=12) moved to the same extent as age-matched controls (N=12), but less than younger individuals (N=12, $P<0.05$). Preliminary exploration of the seven day data suggests that people with Parkinson's move less over the course of the week following attendance at a dance class compared to age-matched controls. Further analyses when data collection is complete will investigate whether this group difference is significant and whether people with Parkinson's are more or less active during the week following a dance class compared with a week when they did not attend a dance class.

Discussion and Conclusions: Wrist-worn accelerometers are a feasible way of tracking the level and pattern of activity made by people with and without Parkinson's over the week following a dance class. Initial results from the seven day study mirror previous research which found that self-reported activity levels by people with Parkinson's were less compared to age-matched controls (van Nimwegen et al.,

2011). Therefore, the devices may be useful as a means of quantifying over the longer term the reduced activity levels that are a characteristic motor feature of Parkinson's.

Acknowledgements: The researcher was funded by a Department of Psychology and Sports Sciences PhD bursary.

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37. EFFECTS OF A THREE-DAY PERIOD OF INTENSE, INTERMITTENT EXERCISE ON OXIDATIVE STRESS AND INFLAMMATION.

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Introduction: It is well documented that strenuous and prolonged exercise induces oxidative stress and inflammation, with the associated muscle damage and fatigue compromising performance. However, little is known about the oxidant effects of intense, intermittent exercise, as performed daily by elite athletes competing in team sports. The purpose of this study was to assess the short-term effects of a 3-day period of intense, intermittent exercise on biomarkers of oxidative stress and inflammation in trained athletes.

Methods: Ten trained athletes (age: 32.11±1.91yrs; mass: 66.33±1.95kg; maximal oxygen uptake (VO_{2max}): 51.44±1.59mL·kg·min^{-1}) completed a high-intensity, intermittent exercise protocol (90-minute intermittent treadmill run, ~70% VO_{2max}) on three consecutive days and were compared to a control group (N=10). Blood samples were collected immediately pre (T1) and post (T2) the 3-day exercise protocol, then 21h- (T3) and 42h-post-exercise (T4); and assayed for Total Antioxidant Status (TAS), Thiobarbituric Acid Reactive Substances (TBARS), Interleukins (IL-6, IL-8 and IL-10), C-Reactive Protein (C-RP) and Lactate Dehydrogenase (LDH). Data were corrected for plasma volume change; results presented as M±SE.

Results: No significant differences were observed between the exercise and control group at T1 (TAS: 1.20±0.14mmol.L^{-1} vs. 1.18±0.11mmol.L^{-1}; LDH: 302.14±16.24U/L vs. 295.27±31.26U/L; TBARS: 6.21±1.09µM vs. 5.88±1.00µM; and IL-6: 0.67±0.70pg/ml vs. 1.12±0.28pg/ml). The 3-day exercise period caused a significant increase in LDH (413.24±35.27U/L, P = 0.029), IL-6 (2.54±0.35pg/ml, P = 0.037) and TBARS (7.00±0.61µM, P = 0.042) at T2, with the effects of TBARS remaining above baseline at T4 (6.43±0.79µM, P = 0.043). TAS increased post-exercise with a significant difference observed between groups at T2 (1.86±0.21mmol.L^{-1} vs. 1.20±0.13mmol.L^{-1}, P = 0.006), T3 (1.86±0.28mmol.L^{-1} vs. 1.30±0.14mmol.L^{-1}, P = 0.010) and T4 (1.71±0.22mmol.L^{-1} vs. 1.17±0.13mmol.L^{-1}, P = 0.014). IL-8, IL-10, and C-RP did not differ between groups.

Conclusions: A 3-day period of intense, intermittent exercise increased oxidative stress and up-regulated antioxidants in trained athletes, thus confirming the current model that exercise-induced oxidants play an important role in intracellular signalling pathways of endogenous antioxidants.

38. UNDERSTANDING AND PREDICTING PHYSICAL ACTIVITY USING THE COM-B MODEL OF BEHAVIOUR CHANGE

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Introduction: Without a sound theoretical basis providing a rationale for the design of an intervention, as well as criteria for its success, it is difficult to evaluate empirical evidence. An important task for the design of an intervention therefore is to specify the theoretical constructs expected to bring about behaviour changes through clearly stated mechanisms. This study investigated which theoretical domains of behaviour change best formed the capability, opportunity, and motivation constructs of the COM-B model of behaviour change. Additional objectives were to analyse how well these constructs then predicted physical activity, and then to examine motivation as a mediator of the effect of capability and opportunity on behaviour.

Methods: Using a prospective design, 186 healthy adult participants completed measures capturing relevant domains from the Theoretical Domains Framework for the three constructs of the COM-B and then reported their moderate and vigorous physical activity (MVPA) one week later. Eleven of the 14 theoretical domains were measured by validated questionnaires, and the constructs and predictive validity of the COM-B were examined using a formative measurement model.

Results: Action planning, habits, and self-monitoring (behavioural regulation domain) formed capability; Social support and subjective norms (social influences domain) formed opportunity; Self-efficacy (beliefs about capabilities domain), intentions (intentions domain), and exercise self-identity (optimism and social role and identity domains) formed motivation. The COM-B overall strongly predicted MVPA (50% variance explained), with capability and motivation as the key drivers of MVPA. Motivation acted as a weak mediator for opportunity and a strong mediator for capability on behaviour.

Discussion and Conclusions: The COM-B is a useful model to understand and predict MVPA, with capability (psychological) and motivation (reflective) of particular importance. This study also suggests key theoretical domains that behaviour change techniques could target in future physical activity interventions. Future research should consider using this approach to conceptualise the COM-B for use in specific populations and behaviours.

39. A THREE DIMENSIONAL BIOMECHANICAL ANALYSIS OF ATHLETES WITH FUNCTIONAL ANKLE INSTABILITY DURING A DYNAMIC CUTTING MANOEUVRE

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Introduction: Ankle sprains are one of the most common sporting injuries. It has been previously stated that in order to improve preventative measures for ankle sprains a better understanding of the mechanism is needed with biomechanical quantities (Fong, Ha, Mok, Chan, & Chan, 2012). Functional ankle instability (FAI) is defined as 'a history of recurrent ankle sprains and the sensation of giving way' (Tanen, Docherty, Van Der Pol, Simon, & Schrader, 2014). Few studies have used three-dimensional motion analysis for the study of ankle sprains and those that have do not comment upon movement above the tibia. This study will analyse full body biomechanics and compare between those with functional ankle instability and those with no history of ankle sprains during a cutting maneuver.

Aims: To investigate the difference in whole body kinematics of recreational athletes with functional ankle instability in comparison to healthy controls during a cutting manoeuvre.

Method: Nineteen (14 male, 4 female) healthy controls (age 22.4 ± 3.6, height 177.8 ± 7.6, mass 70.4 ± 11.9), and 19 (13 male, 5 female) athletes with functional ankle instability (age 22.0 ± 2.7, height 176.8 ± 7.9, mass 74.1 ± 9.6) kinematics were analyzed in this study. In the FAI group, the unstable ankle and stable ankle were identified using the identifying functional ankle instability questionnaire. In the control group the dominant and non-dominant were distinguished by asking which leg they would use to kick a ball. Three-dimensional motion analysis data was recorded using the Owl Digital Real Time 10 Camera System (Motion Analysis, Santa Rosa, California). The Helen Hayes marker set and a modified oxford foot model were combined to digitise points across the whole body. Electromyographic data was recorded bilaterally for the gluteus medius, tibialis anterior and the peroneus longus. Participants performed 3 cutting manoeuvres for each foot from a land to standardise the approach speed. This simulated a sport specific mechanism for an ankle sprains. In order to avoid focus bias statistical parametric mapping was used for the statistical analysis. This will enable curve analysis for the trial duration for the trunk, hip, knee, ankle and foot in all 3 planes of motion. Muscle activation will also be analyzed.

Expected outcomes: Healthy athletes are thought to utilize an ankle strategy in maintaining the bodies' centre of gravity over the base of support by pronating and supinating the foot. However it has been postulated that athletes with functional ankle instability utilize a hip strategy due to changes in central neural control with the presence of ankle joint dysfunction. It is therefore hypothesized that the group with functional ankle instability will recruit a hip strategy to maintain their centre of gravity whilst the healthy group will use an ankle strategy.

MICROBIOLOGY, MOLECULAR BIOLOGY & BIOTECHNOLOGY

40. STRUCTURAL AND FUNCTIONAL STUDIES OF THE ORF1 PROTEIN FROM THE INSERTION-SITE SPECIFIC RETROPOSON M5 FOUND IN INDO-PAKISTAN URBAN MALARIAL VECTOR *ANOPHELES STEPHENSI*

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Introduction: In the 1950s Barbara McClintock inferred the occurrence of transposition: the movement of small segments of DNA - entities known as transposable elements from one position of the genome to another. Classification of transposable elements in regards to mechanism of transposition distinguishes them into two groups; **transposons (Class II)** and **retroposons (Class I)**. The term retrotransposon was coined as it illustrates the transposition of these elements is dependent on the reverse transcription of RNA to DNA through a reverse transcriptase, also known as the 'copy and paste' transposition. The M5 retrotransposon has been found in numerous mosquito species such as *Anopheles stephensi.* M5 present in these *Anopheles* is a class 1, non-LTR transposable element of the jockey clade family with two open reading frames (ORF). Due to its APE like endonuclease, M5 should transpose to random sites of the genome. However, in *A. stephensi* the element has been reported to transpose with site specificity. The aim of the project is to gain structural and functional information on the role of the ORF1 protein of M5 in order to understand the element's site specificity.

Methods: To perform functional and structural studies, an *Escherichia coli* expression vector was designed with a synthetic AsM5 ORF1 insert. Heterologous expression of ORF1p in *E.coli* was done by activating the *Lac* operon in the vector using IPTG as an allactose substitute. Immobilized metal ion affinity chromatography (IMAC) was used to purify the protein using its polyhistidine tag as a target. *Saccharomyces cerevisiae* was also chosen as a potentially suitable expression system for this protein. The AsM5 ORF1 gene was cloned from the *E.coli* expression vector into a cloning vector before being sub cloned into the pYES *S.cerevisiae* vector, using the Xba1 and EcoR1 restriction sites. The expression of ORF1p is induced by adding galactose and activating the *GAL1* promoter on the vector. Trial expressions are underway and cell lysates are examined using SDS PAGE and Western blotting before bulk expression and purifications.

Results: Expression of ORF1p in *E.coli* and 'his-trap' purifications yielded protein bands that either showed degradation or very low yield of the unfolded protein. Sequencing verified that the AsM5 ORF1 insert was successfully cloned into the pYES vector. Initial expression trails have shown promising data in the form of a protein band at 55kDa on SDS PAGE. Nonetheless, more specific techniques such as a Western blotting are needed to better confirm protein expression.

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41. DEVELOPMENT OF MYCOVIRUS BASED VECTORS TO SILENCE DOTHISTROMA SEPTOSPORUM GENES

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Introduction: Mycoviruses specifically infect and replicate in fungi, they are cryptic in nature and usually associated with hypovirulence (reduced virulence) or more unusually hypervirulence. It is suggested that, with the discovery of mycoviruses in Aspergillus fumigatus, hypovirulence can be induced through gene silencing. Dothistroma septosporum is the causal agent of Dothistroma needle blight affecting Pinus *spp*. Its origin is unclear with suggestions that it originated from South America and Nepal, with early reports in 1911 in Eastern Europe and in the 1920's in Western Europe and North America. Following a screen of over 49 isolates we discovered one isolate infected with a tetra partite mycovirus reminiscent of a chrysovirus which we have named Dothistroma septosporum chrysovirus (DsCV-1), belonging to the family Chrysoviridae. DsCV-1 shares similar phenotypic and genomic properties to Aspergillus fumigatus chrysovirus possessing a tetra partite, double-stranded RNA (dsRNA) genome, RNAs 1-4 with respective accession numbers (FN168512, -13, -14 and -15) and sizes 3560, 3159, 3006 and 2863 bp. The aim of this project is to construct full-length clones of DsCV-1 characterising in detail the proteins involved in gene silencing which is currently underdeveloped in fungi and to construct gene silencing vectors.

Methods: At this early stage of the project, approximately 57% of the tetra partite DsCV-1 dsRNA genome has been cloned and sequenced using genome walking and RLM-RACE recombinant DNA technology.

Results: 1114 amino acids of RNA 1(FN168512), 953 amino acids of RNA 2 (FN178513), 977 amino acids of RNA 3(FN178514) and most recently 1405 amino acids of RNA 4(FN178515). Have been cloned using recombinant DNA technology presently at this early stage of the project

Discussion: Due to the early stages of this project at moment, the results achieved are highly promising but inconclusive.

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42. THE EFFECTS OF A PROBIOTIC (YAKULT, *LACTOBACILLUS CASEI* SHIROTA) ON THE GUT MICROBIOTA

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Introduction: *Clostridium difficile,* is often identified in patients with antibiotic-associated, hospital-acquired diarrhoea. In 95% of cases of pseudomembranous colitis, the presence of *C. difficile* is observed. Some antibiotics compromise the protective effect of the microflora, thus providing a suitable environment for the proliferation of pathogens. Probiotic therapy with lactobacilli has been shown to reduce the likelihood of CDI. Notwithstanding, the beneficial effects of probiotics in treating/preventing CDI are still being debated due to poorly designed clinical studies.

Objective: The effects of a probiotic (Yakult, *Lactobacillus casei* Shirota) on the gut microbiota were investigated in a clindamycin-dosed *in vitro* triple stage chemostat model of the human colon.

Methods: Faeces from healthy elderly volunteers (N=5) was screened for the presence of *Clostridium difficile* following anaerobic culture on Brazier's agar for 48 hours. Faeces were then pooled and used to create a 10% emulsion in pre-reduced PBS. The faecal emulsion was used to inoculate the gut model. The flow rate of growth medium in the gut model was 13.2ml/h (D=0.015 h^{-1}). The gut model microflora populations were allowed to equilibrate for 14 days and the concentrations of the main cultivable members of the indigenous gut microbiota were ascertained by viable counting on selective and non-selective agars. These bacterial groups included: total bacteria (facultative + obligate anaerobes), *Bacteroides fragilis* group, *Bifidobacterium* spp., total *Lactobacillus* spp., lactose fermenting Enterobacteriaceae*,* and *Enterococcus* spp. A probiotic drink containing 6.4 x 10^9 CFUs *L. casei* Shirota per bottle was administered daily into vessel 1 of the gut model. On day 22, Clindamycin concentrations equivalent to levels expected in the faeces (33.9 mg/L) of patients were instilled into an *in vitro* gut model 4-times daily for 7 days. *L. casei* Shirota was dosed for first 42 days, following which probiotic dosing ceased and gut microflora populations were monitored for another 14 days .

Results: *L. casei* Shirota administration lead to a decline in the lactose-fermenting Enterobacteriaceae and enterococci populations by approx. 2 $\log_{10}$ cfu/mL. Bifidobacteria viable counts increased as lactobacillus counts increased. Clindamycin instillation elicited significant declines in bifidobacterial populations (approx. 6 $\log_{10}$ cfu/mL), which were still within the limits of detection. *Lactobacillus* spp. counts declined by approx. 4 $\log_{10}$ cfu/mL during clindamycin instillation, and a less prominent decline in *Bacteroides fragilis* group viable counts was observed. However, enterococcal and total anaerobe viable counts increased by approx. 1 $\log_{10}$ cfu/mL and 2 $\log_{10}$ cfu/mL respectively. Additionally, lactose-fermenting Enterobacteriaceae counts increased by approx. 3 $\log_{10}$ cfu/mL.

Conclusions: Antibiotic treatment substantially altered the composition of the microbiota and the effects of clindamycin instillation reflected prior *in vitro* and *in vivo* studies. However, the LcS counts declined upon

cessation of its dosing, indicating a poor colonisation and possessing short term benefits. To confirm these findings, and evaluate the potency of probiotics, more studies need to be carried out.

43. WHAT IS THE QUALITY OF ONLINE INFORMATION ABOUT DIET AVAILABLE TO PEOPLE WITH TYPE 2 DIABETES?

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Introduction: People with Type 2 Diabetes (T2DM) need to be able to access good quality information in order for them to be able to manage their condition (Weymann et al., 2015) and to access accurate and up-to-date information about diet. Research suggests that most people with T2DM use the internet on a regular basis to find out about how to manage their condition. In addition research has shown that dieticians recommend their patients with T2DM to review specific websites (McClinchy et al., 2016). Research utilising quality assessment tools that were developed to assess the quality of online information suggests that the quality of online health information accessed by people with T2DM is variable (Weymann et al., 2015, Charnock et al., 1999).

Aim: The aim of this study was to compare the quality of information about diet found on websites that were likely to have been accessed by people with T2DM with that found on websites recommended by dieticians to people with T2DM.

Methods: Ten websites that were identified using the search term 'type 2 diabetes what should I eat' and ten websites that were recommended by dieticians to people with T2DM identified in previous research (McClinchy et al., 2016) were selected for analysis. Weymann's quality criteria (Weymann et al., 2015) and the tool DISCERN (Charnock et al., 1999) were used to assess the quality of the information in the identified websites.

Results: Only seven from the ten websites on the dieticians' recommended list (DRL) could be located. Both tools found similar overall agreement with the criteria. The Weymann tool had 61% agreement with quality criteria for the websites identified from the patients' search (PS) and had 65% agreement with the quality criteria for the websites identified on the DRL, while the Discern tool had 65% agreement for the PS and 64% for the DRL. The lowest score was 31% with the Weymann tool and 33% with the Discern tool for a blog website (www.joybauer.com) identified from the PS. The highest score was for the NHS choices website www.nhs.uk, identified on both the PS and the DRL achieving 88% with the Weymann tool and 83% with the Discern tool. The lowest scoring website on the DRL was a charity diabetes education website (http://www.xperthealth.org.uk/) which scored 48% with the Weymann tool and 56% with the Discern tool.

Discussion: Despite their development being 15 years apart both tools identified similar levels of quality across the two groups of websites with both identifying a blog website from the PS having the lowest score. Not all websites that had been recommended to patients by dieticians that had been accessible the previous year were still accessible. The range of quality was greatest in the PS which also included the

website with the lowest score. However the PS included the highest scoring website that had also been identified on the DRL.

Conclusion: People with T2DM are able to use effective searching methods to find information online about what to eat however they may need assistance from healthcare professionals in the identification of sources which are of the highest quality.

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44. HEMIN EFFECT ON REDUCED SUSCEPTIBILITY TO METRONIDAZOLE IN EPIDEMIC *CLOSTRIDIUM DIFFICILE* CLINICAL ISOLATES

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Introduction: *Clostridium difficile* has been observed to be the main aetiological agent of pseudomembranous colitis and a major cause of antibiotic associated diarrhoea. Resistance to a range of antimicrobials has been reported in *Clostridium difficile*. Additionally, reduced susceptibility to one of the frontline treatment antimicrobials, metronidazole, has been observed. This research was aimed at detecting the effect of hemin on the reduced susceptibility to metronidazole (MTZ) phenotype in clinical *C. difficile* isolates from the UK.

Methods: The effect of hemin on MTZ susceptibility in *C. difficile* strains of ribotypes 001, 027 and 106 was examined using agar incorporation MIC method with both supplemented Brucella and Wilkins Chalgren agars (BBA and WCA). A Man-Whitney test of $\log_2$-transformed MICs was used to detect the significance of the difference observed in MICs due to 5mg/L hemin supplementation.

Results: On Wilkins Chalgren Agar, MTZ MIC stability was same with or without hemin with the exception of 3 strains (027 ribotype n=2, and 001 ribotype n=1) which had +/- 1 dilution difference in MIC. *C. difficile* PCR ribotype 001 MTZ MICs were significantly elevated in the presence of hemin using BBA (P = 0.021); this effect was also observed for PCR ribotype 027 (P = 0.029).

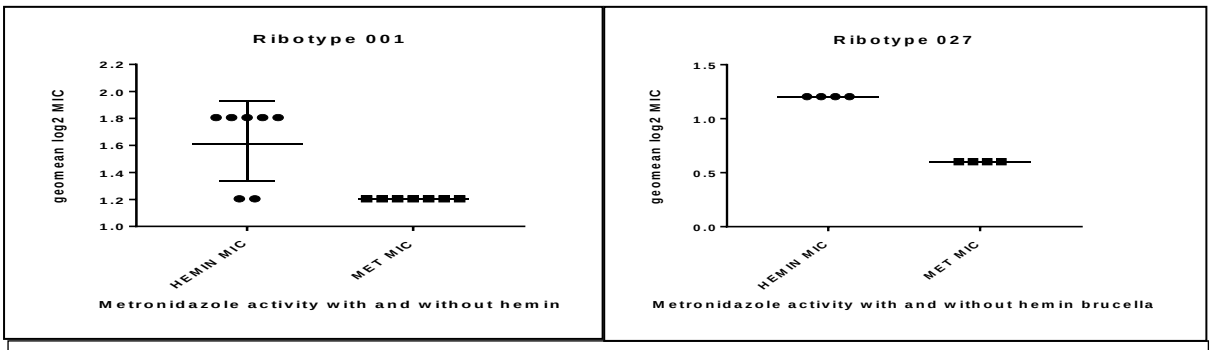

Figure 1: Effect of hemin supplementation (5mg/L) on MTZ suceptibility in brucella agar in *C. difficile* PCR ribotypes 001 and 027 using an agar incorporation MIC method.

Discussion and Conclusions: Supplementation of WCA with additional hemin did not alter MTZ MICs. However, 63% of all reduced susceptible strains tested showed declines in MTZ MICs without hemin in BBA. Additionally, all of *C. difficile* PCR ribotype 027 strains showed declines in MTZ MICs without hemin in BBA, thus confirming hemin enhances the stability of the reduced susceptible MTZ phenotype in *C. difficile* PCR ribotypes. The mechanism by which this occurs is yet to be confirmed and further research using NGS is required in order to study the reduced susceptibility to MTZ phenotype.

45. TRACKING THE METABOLIC SIGNATURES ASSOCIATED WITH THE FATAL CHILDHOOD MOTOR NEURON DISORDER SPINAL MUSCULAR ATROPHY

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Introduction: Spinal muscular atrophy (SMA) is an inherited neurodegenerative disorder that is the leading genetic cause of infant mortality. SMA primarily targets alpha motor neurons in the anterior horn of the spinal cord leading to gradual muscular weakness, paralysis and ultimately death due to respiratory defects (Lefebvre et al., 1995). SMA is caused by homozygous deletions or loss of function mutations in the telomeric copy of the *survival motor neuron 1* (*SMN1*) gene. *SMN2,* its duplicated centromeric copy, remains intact and acts as a disease modifier gene, since it can modulate SMA severity in a copy-dependent manner. *SMN2* cannot compensate for the loss of *SMN1* due to a C>T nucleotide change in exon 7 that affects *SMN2* pre-mRNA splicing and results in a truncated protein of diminished function and stability (SMNΔ7). The scientific community still does not understand why decreased function of the SMN protein causes selective neurodegeneration, nor is there a consensus on the cellular and molecular pathways that are critical for SMA pathology. Recent studies have proposed that a metabolic disruption may be the cause of motor neuron death in SMA (Miller et al., 2016). Furthermore, parallels can be drawn between SMA and other motor neuron diseases such as amyotrophic lateral sclerosis (ALS), where energy metabolism defects have been identified. Here we assess the metabolic differences between SMA patient fibroblasts and healthy controls to discern whether these changes can be exploited for future therapies.

Methods: Seahorse Biosciences XF assay will be utilised to measure the changes in extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), which can gauge cellular metabolism. This allows for the comparison of metabolic profiles between SMA derived cells and healthy controls. Once these pathways have been identified, liquid chromatography-mass spectrometry (LC-MS) will also be utilised to identify specific metabolite alterations in disease fibroblasts for further investigation into precise metabolic changes in SMA.

Results and Conclusions: Currently, control and patient fibroblasts are being cultured and stocked. Cell lysates have been isolated for Western blot analysis of endogenous SMN levels. We hypothesise that SMN depletion will have an effect on mitochondrial bioenergetics, possibly resulting in increased oxidative stress and reduced mitochondrial respiration represented by a reduction in OCR. Furthermore, metabolic parallels between ALS and SMA may arise such as increased energy expenditure, glucose intolerance, and a metabolic dependency switch, for example from glucose to fatty acid metabolism as a source of energy.

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46. EVOLUTIONARY TARGETED DISOVERY OF INFLUENZA A VIRUS REPLICATION INHIBITORS

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Introduction: Influenza A is an infectious virus causing significant respiratory illness. In addition to annual epidemics, serious outbreaks have been reported in previous years such as the 1918 Spanish flu killing over 40 million people. Upon infection, antiviral drugs targeting the neuraminidase surface protein and M2 transmembrane protein are the only treatment options available. However, rapid mutation rates and emergence of antiviral drug resistance are concerning. To address this problem and the lack of effective drugs available this research aims to identify 1) highly conserved regions of Influenza A viral proteins that overlap with potential ligand binding sites and 2) inhibitor molecules that can bind to those sites.

Methods: Protein sequences of the polymerase basic protein 2 (PB2) were obtained from the NCBI Influenza virus resource and aligned using Clustal Omega. The degree of amino acid conservation was calculated based on Valdar's scoring method. Missing parts of the experimental structure were predicted using a state-of-the art protein structure prediction method. Ligand binding hot spots were identified with computational solvent mapping based on the FTMap algorithm. A selected hot spot was subjected to virtual screening against a library of ~50,000 chemical compounds from the National Cancer Institute library and 1738 FDA approved drugs from the DrugBank library using AutoDock Vina.

Results: Conservation analysis based on 12,459 PB2 sequences confirmed the overall protein structure is highly conserved, whilst individual regions of low conservation were located on the exterior of the protein structure. Fifteen binding hot spots were predicted in different PB2 sub domains; some of which reside in areas of unknown function. Virtual screening showed binding affinities of up to -10.3 kcal/mol. Top molecules were found to interact with conserved residues including Gln138, Gly222, Ile539, Asn540, Gly541, Tyr531 and Thr530. From the DrugBank library Paliperidone was predicted as a top hit drug.

Conclusions: Potential binding sites of high conservation were identified that could be further investigated to identify novel interactions between PB2 and other proteins or cellular metabolites. The predicted drug-like compounds could serve as laboratory tools to investigate PB2 functions and/or be developed into antivirals.

Acknowledgements: We thank Mr Jamie Stone for providing technical assistance. This work has made use of the University of Hertfordshire high-performance computing facility. The research studentship was funded by the School of Life & Medical Sciences.

47. THIAMINE CONTENT OF PULSES PROCESSED WITH OR WITHOUT SULPHITES

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Introduction: Pulses are an important source of protein, carbohydrates, fibre, vitamins, minerals and phytochemicals for many people worldwide. Due to the lengthy cooking and processing time of dried pulses required to make these foods more palatable, many will choose to purchase them canned. In some canned pulses, sodium metabisulphite is added. However, this preservative also degrades thiamine, a vitamin found in many varieties of beans. The aim of this study is to investigate if sulphites affect the total phenolic content (TPC), active thiamine content (ATC), and antioxidant capacity (AOC) of chickpeas (*Cicer arietinum* L.) and butterbeans (*Phaseolus lunatus* L.).

Methods: TPC (Folin-Ciocalteu assay), AOC (Ferric Reducing ability of Plasma (FRAP) assay) and ATC (VitaFast Microbial assay) were measured in eight different samples of chickpeas and butterbeans (with or without added sulphites). Assays were carried out in triplicate, and each experiment was duplicated.

Results:

Table 1: Total phenolic content (TPC), antioxidant capacity (AOC) and active thiamine content (ATC) of pulses (mean ± SD).

	Chickpeas			**Butterbeans**		
	With sulphites	Without sulphites	*P value*	With sulphites	Without sulphites	*P value*
TPC (mg GAE/100g)	71.51±33.11	58.57±28.14	0.166	25.81±5.84	29.64±14.67	0.125
AOC (µmol $FeSO_4$ Eq./100g)	377.04±71.17	357.41±69.10	0.257	1116.90±131.8	976.47±209.63	0.001*
ATC (mg active thiamine/100g)	0.02±0.01	0.19±0.12	<0.001*	0.07±0.28	0.24±0.09	0.005*

*With sulphites significantly different from without sulphites. All samples are given as 100g of dry weight (DW) sample.

There was a significantly higher ATC in chickpeas and butter beans not containing sulphites than in these pulses containing sulphites ($p<0.001$) (Table 1). Sulphite did not significantly influence TPC in chickpeas or butterbeans ($p>0.05$) (Table 1). Sulphite did not significantly affect AOC in chickpeas ($p>0.05$), but there was a significantly higher AOC in butterbeans containing sulphites compared to those not containing sulphites ($p<0.005$) (Table 1).

Conclusions: This study found that chickpeas and butterbeans containing sulphites have significantly lower thiamine levels than products not containing sulphites. As these and other pulses are a good source of thiamine, consideration should be taken when adding sulphites to canned pulses as this may impact on the overall nutritional value of the meal. This is especially important for those individuals who do not

consume other sources of thiamine such as pork, due to ethical or religious reasons. Further investigations are warranted to establish if adding a food containing sulphites may impact the thiamine levels of the rest of the meal.

Acknowledgements: We would like to thank the Perry Foundation, the Centre for Research in Primary and Community Care (CRIPACC) and the Centre for Agriculture, Food and Environmental Management (CAFEM) for funding.

48. SYNTHESIS, ANTIMYCOBACTERIAL ACTIVITY EVALUATION AND MODE OF ACTION OF SERIES OF NOVEL DIKETONES

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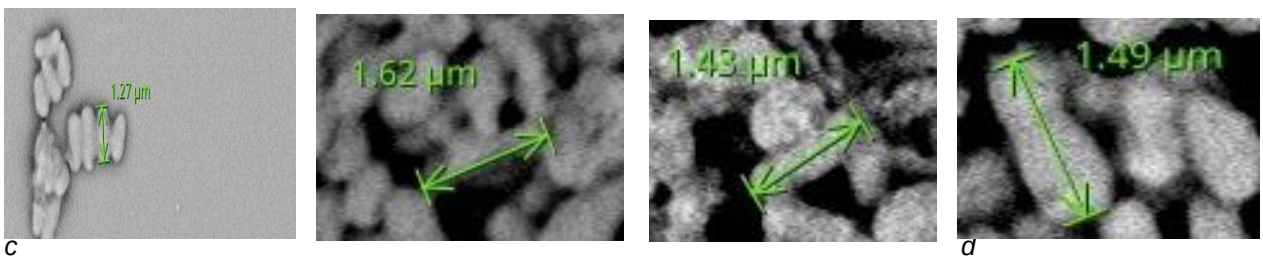
Background: The emergence and development of drug-resistant strains of bacteria are increasing threat to the society. More than 2 million people in the US alone become ill every year due to antibiotic-resistant infections and 23,000 die from such infections. In the EU, 25,000 patients die annually as a result of infections caused by resistant bacteria. The WHO estimates that 9 million people developed active tuberculosis in 2013 and 1.5 million people died from it. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis continue to spread worldwide with an estimated 480,000 new cases in 2013. Emergence of XDR-TB and TDR-TB has emphasised the necessity for development of new drugs that can effectively combat these newly evolved resistant strains of *M. tuberculosis*.

Material/methods: A series of 17 novel diketones were synthesised using substituted acetophenones and benzoyl chlorides by the Baker Venkataraman Reaction and were characterised using spectroscopic techniques IR, LC-MS, ^{1}H and ^{13}C-NMR. The minimum inhibtory concentration (MIC) of the synthesised compounds was evaluated against *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG using broth micro-dilution assay according to CSLI guidelines. The *in vitro* cytotoxicity of the synthesised compounds was evaluated against murine macrophage J774 cell line by performing MTT assay. Scanning electron microscopy was performed to investigate the mode of action of the lead compound by treating the cells with 10 X MIC of the lead compound and Isoniazid.

Results: Two compounds 02 and 03 were found to have activity against *Mycobacterium bovis* BCG with MIC of 1 µg/ml and 2 µg/ml respectively. Compound 02 and compound 03 exhibited IC_{50} values of 23 µg /ml and 35 µg /ml respectively against J774 cells. The preliminary examination of Scanning Electron Microscopy (SEM) clearly shows the morphological changes in the cells that were treated with compounds 02 and 03, figure1. The whole genome sequencing will be performed to further confirm the mode of action.



c *d*

Figure 1: **Scanning electron micrographs of *M. bovis* (BCG) cells**: a. Control; b. treated with Isoniazid for 24hours; c. treated with compound 02 for 24 hours; and d. treated with compound 03 for 24 hours.

Conclusions: The synthesised compounds are found to be active against *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG. Further studies on mode of action and structure activity relationships will allow the development of more potent active compounds.

49. EXPLORING THE ENDOSPORE SURFACE COAT OF *PASTEURIA PENETRANS*, THE BACTERIAL HYPERPARASITE OF PLANT-PARASITIC NEMATODES, USING *BACILLUS* SPP.

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Introduction: *Pasteuria penetrans,* previously named as *Bacillus penetrans,* belong to a group of Gram positive endospore forming bacteria (phylum: *Firmicutes*; family: *Pasteuriaceae*) that are hyperparasites of plant-parasitic nematodes. These bacteria could provide an environmentally benign and sustainable control strategy for economically important plant-parasitic nematodes as an alternative to nematicides. Earlier studies based on *16S rRNA*, *spoOA* and other housekeeping genes suggest a close relatedness of *Pasteuria* to *Bacillus*. They are obligate parasites, but recently they have been mass produced by a proprietary fermentation technology owned by Syngenta. The attachment of *Pasteuria* endospores to the cuticle of their host nematode is a crucial step in the infection process. The molecular structure of the *Pasteuria* endospore is little studied, although collagen-like fibres present on the spore surface are thought to be involved in spore attachment to its host. Some 4000+ genome survey sequences of *Pasteuria* are available in public databases, but there is not as yet a complete annotated genome. In the current study, we utilize well characterized endospore forming *Bacillus* spp. as comparative tools to investigate the surface of *Pasteuria* endospore.

Methods: Sequenced genomes of animal pathogenic and non-pathogenic *Bacillus* spp. were used to identify selected genes related to endospore and exosporium formation and function. Synteny across the selected genes was explored using a web-based synteny explorer software SyntTax (Oberto, 2013). The genome survey sequences (GSS database, NCBI) of *Pasteuria* were interrogated for the presence of sequences homologous to selected *Bacillus* genes. Antibodies raised against *Pasteuria* endospores show cross-reactivity to proteins extracted from endospores of *Bacillus thuringiensis* following Western blot analyses. Subsequent immunofluorescence microscopic comparisons of *Pasteuria* and *Bacillus thuringiensis* were performed using *Pasteuria* specific antibodies.

Results: *In silico* studies identified the presence of at least 10 collagen-like coding genes in the *Pasteuria* genome. Western blots indicate similarities in the endospore protein profile of *Bacillus* and *Pasteuria.* Likewise, fluorescent labelling of endospores coats using polyclonal antibodies showed similarities in the antigenic determinants of *Pasteuria* and *B.thuringiensis.* Further characterisation of these determinants suggests the involvement of carbohydrate moieties.

Discussion: This study is a step forward in our understanding of the *Pasteuria* endospore surface properties. The similarities in the endospore surface biochemistry of *Pasteuria* to that of *B.thuringiensis* can

be further exploited to study the endospore heterogeneity in *Pasteuria.* This will help us to better our understanding of the molecular mechanisms governing the *Pasteuria*-nematode interaction.

GEOGRAPHY, ENVIRONMENT & AGRICULTURE

50. REDUCING EPIDEMICS OF STRAWBERRY POWDERY MILDEW USING A SILICON NUTRIENT TO GIVE REDUCED SUSCEPTIBILITY TO *PODOSPHEARA APHANIS*

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Introduction: The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by the fungus *Podosphaera aphanis,* which has to be controlled by the frequent use of fungicides (Dodgson, Hall & Parker, 2008). Work carried out at the University of Hertfordshire has shown that the weekly use of a silicon nutrient in the fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease. The work reported here aims to quantify this effect.

Methods: The effect of a silicon nutrient in reducing susceptibility to strawberry powdery mildew was monitored fortnightly in a commercial polythene tunnel from April until September 2016 at Maltmas Farm, Wisbech. The trial was in 5 beds in one tunnel. Ten leaves from each of 5 beds were sampled from each treatment at each sample time. The silicon nutrient (Sirius) was delivered once or twice a week through the fertigation tubes according to the treatment at a concentration of 0.017%. The treatments were, the control (untreated) - no silicon nutrient and no commercial fungicides; silicon nutrient and commercial fungicides; silicon nutrient only; silicon nutrient twice a week plus commercial fungicides and silicon twice a week only. Samples collected were assessed in the laboratory by the percentage of mycelium coverage per leaflet. The epidemic build-up recorded was for 12 weeks, which started in June and monitoring stopped on September 6 when harvest ended.

Results: The results showed that the untreated (no silicon no fungicides) plants had an average of 61% disease coverage of mycelium per leaflet, silicon plus fungicides had 43% disease coverage of mycelium per leaflet, silicon plus no fungicides had 21% disease coverage of mycelium per leaflet, silicon twice plus fungicides had 6% disease coverage of mycelium per leaflet and silicon twice with no fungicides had 5% disease coverage of mycelium per leaflets.

Conclusions: This shows that the addition of a silicon nutrient via the fertigation tubes reduced susceptibility to the disease and thus reduced disease severity.

Reference:

Dodgson J, Hall A, Parker S. (2008). Rule based prediction system to optimize fungicide applications for control of Podosphaera aphanis. In VI International Strawberry Symposium 842 Mar 3 (pp. 355-358).

51. THE HOUSING EXPERIENCES AND LONG-TERM INTENTIONS OF POLISH MIGRANTS IN LUTON, CENTRAL BEDFORDSHIRE

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Introduction: The significant increase of Eastern European migrants in the UK following EU expansion in 2004 has had a substantial short-term impact upon UK public and private services, whilst the long-term impacts remain to be seen (Glasgow Housing Association, 2008). It is widely recognised that housing is one such service that has experienced a significant increase in demand as a direct result of migration, yet whilst there have been some important UK based studies regarding the relationship between internal migration and housing (e.g. Coulter, Van Ham & Findlay, 2013), research in this area has slowed in the UK since the mid-1990s (Smith & Finney, 2015). Amas (2008) suggests that the impact of the influx of new migrants into a locality has been under researched and describes a lack of in-depth studies regarding the housing experiences of new labour migrants. This presents a challenge for local councils and planners in mapping out future service provision and in ensuring an integrated, connected community. Therefore, the research project could assist to close this gap in the knowledge. Furthermore, the uncertainty that has arisen from Brexit and the large amount of hate crime that has ensued may pressure EU migrants to return back to their countries of birth, possibly acting as a release valve to the high level of inward European migration to the UK. The data collected in this study could increase the understanding of the views and experiences of Polish migrants in the UK post-Brexit and could also help to monitor Polish migrant's propensity for movement following the referendum result.

Methods: To collect data on the Polish migrant experience in the UK, interviews will be carried out with Polish migrants living in Luton (an *entrepôt* location), Peterborough and Ealing, all of which have particularly large concentrations of Polish residents. In addition, the research will incorporate the reflections of a range of Polish nationals currently living in Poland, including those who have ambitions to live and work in the UK and those who have done so and returned, in order to understand transnational ties and the full range of factors that influence the decision-making and behaviour of Polish migrants. Additionally local housing experts in Luton, Peterborough and Ealing will be interviewed to provide further information about the housing market in these localities and their views on how Polish migrants negotiate housing in those areas. Furthermore, observational data will be collected periodically from each study area in order to add further context and value to the study.

Results: Data analysis is ongoing and there are only preliminary findings to date.

Conclusions: The findings of this research will have potentially wide-reaching impact. The research will significantly extend the current knowledge of the housing experiences of new economic migrants, with a particular focus on those from Poland by way of example. This will provide additional insight of significant value to council and town planners at a time when housing supply is under immense pressure across the UK.

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Smith, D. P., & Finney, N. (2015). Housing and internal migration. *Internal Migration: Geographical Perspectives and Processes*, 81-98.

52. THE ROLE OF BRASSICA PLANT SURFACE CHARACTERISTICS IN RESISTANCE AGAINST THE LIGHT LEAF SPOT PATHOGEN *PYRENOPEZIZA BRASSICAE*

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Introduction: The disease Light Leaf Spot is caused by the pathogen *Pyrenopeziza Brassciae* and is the most important disease affecting oilseed rape, causing up to £150 million losses per year across England and Wales (www.cropmonitor.com). The early stages of colonisation of the pathogen are asymptomatic, making applications of fungicides difficult to time. Additionally, there is wide-spread insensitivity to various fungicides and breakdown of cultivar resistance genes. This project will focus on the role of plant surface characteristics in resistance against *P. brassicae* colonisation. It aims to discover whether the specific composition and type of epicuticular waxes of a plant give certain cultivars greater resistance to *P. brassicae*, and whether different plant tissues will affect pathogen propagation.

Planned Work: The significance of leaf surface characteristics in conveying resistance. Using a mini set of oilseed rape cultivars utilised in an ERA-CAPS project (MAQBAT, www.eracaps.org), plus additional cultivars chosen for their interesting phenotypes, each cultivar will be inoculated. The light leaf spot disease will be assessed over the course of a month, using DNA quantification, and visual assessments of % leaf area covered by P. brassica sporulation at later stages. The most resistant and the most susceptible cultivars will be analysed using different methods for cuticular wax quantification and composition.

Host and non-host resistance in Brassicas. *Brassica napus* and other closely related plant species within the Brassicaceae family will be inoculated with *P. brassicae* to compare responses and discover potential non-hosts of the pathogen. Consulting a phylogenetic tree of the Brassicaceae family will highlight plant species that are closely related to *Brassica napus*. Selected species will undergo the same experiments as conducted with oilseed rape. The varying disease phenotypes on the leaves of these plant species will be compared with the responses of oilseed rape, a known host. A lack of disease phenotype on related plants will indicate a non-host species. The host and non-host results will be used to investigate potential characteristics that differ in non-host plants and that can be utilised to provide greater resistance in host plants.

Susceptibility of different plant tissues. Different plant tissues of oilseed rape will be inoculated at designated points on the plant using paper discs soaked in *P. brassicae* conidial suspension in an initial glasshouse experiment. Different areas on the leaves, various points along the stem, the seed pods, and flowers will all be inoculated. The plants will be grown to full maturity then harvested, so each plant tissue can be inoculated ex planta. Use of microscopy, trypan blue leaf staining, and pathogen quantification (qPCR) will give an insight into the pathogen activity on the surface of the organs and inside the plant tissue in relation to the amount of *P. brassicae* present.

Induced defence. By quantifying oilseed rape callose deposition and lignification when challenged with *P.brassicae* and comparing with the resistance found at plant surface level, I aim to analyse the relationship between these two modes of defence. This, along with information that will be obtained from work on the MAQBAT Project (ROS Assay), will provide an overview for PAMP responses.
This project will provide the basis for future work to exploit the surface characteristics of Brassica species to prevent disease and subsequent damage to crops, which will lead to an increase in annual crop yield and a decrease in economic losses.

53. FOOD POLICY: THE STATE AND PROMOTION OF BRITISH FOOD

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Introduction: In recent years UK food policy has shifted its priorities from a more integrated approach to achieving environmental and public health goals to a greater specific focus on the promotion of the British food industry in the national and international market places. With Brexit imminent and global food security high on the political agenda, a more detailed and critical analysis of the policy shift towards promotion of British food is particularly timely.

Methods: The theoretical study reviews literature on the role of the state in promoting and ensuring economic competitiveness, e.g. the 'Competition State' thesis (Cerny, 2010) and entrepreneurial vision, e.g. the 'Entrepreneurial State' thesis (Mazzucato, 2014). This will theorise a better understanding of actions pursued by the contemporary UK state in relation to the promotion of British food products and producers.

The empirical study analyses the policy advocacy and views adopted by different agricultural and food industries, representation bodies together with other stakeholder voices such as trade interests and small-scale speciality food producer groups. The framing of British food around international export markets, industry entrepreneurialism, quality, authenticity and provenance is being analysed. The development of the role of the state and of different Government departments, agencies and their interactions, along with attendant stakeholders will be applied to recent and current policy initiatives. Policy initiatives of interest include *The Great British Food Unit, British Protected Food Names and the Government Buying Standards for Food and Catering Services*.

To contextualise discussions, a longer-term historical view critically examining the development of the UK state as a food growing, food trading nation as well as how its approaches to the national and international food supply have been articulated and performed are explored. Policy documents and reports (Government & stakeholders) will be analysed to identify policy discourse, advocacy positions and institutional processes by which they have been translated into policy. Stakeholder and elite policy participant interviews will be undertaken to supplement empirical data from written and recorded evidence.

Results: Changes within UK Government approaches to food policy and the promotion of British food have been identified pre and post 2010.

Discussion: This research study aims to apply theories of state policy actions in an era of global economic liberalisation (notably the Competition State and the Entrepreneurial State) to understand the selection and development of public policies to promote the interests of British Food products and producers.

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Cerny, P. G. (2010). *Rethinking world politics: a theory of transnational neopluralism.* Oxford: Oxford University Press.

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54. MONITORING THE REGIONAL DISTRIBUTION OF RACES OF *LEPTOSPHAERIA MACULANS* POPULATIONS IN THE UK

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Introduction: Phoma stem canker, caused by the fungal pathogen *Leptosphaeria maculans*, is a damaging disease on oilseed rape in the UK and can cause yield losses up to 50% if the disease is not controlled. Currently, this disease causes UK annual yield losses >£100M despite use of fungicides. With the recent loss of some effective fungicides (e.g. Punch C) and likely loss of more fungicides through EU legislation, and predicted global warming, yield losses are likely to increase. Use of durable host resistance to control this disease is becoming ever more important. Resistance against *L. maculans* relies on major resistance (*R*) gene-mediated resistance and quantitative resistance. *R* gene-resistance against *L. maculans* is race-specific and is associated with a gene-for-gene interaction. *R* gene-mediated resistance is often rendered ineffective in 2-3 years due to *L. maculans* population changes from avirulent to virulent. This causes losses not only to UK farmers but also to UK breeders (loss of germplasm when resistance breaks down). Therefore, detection and identification of virulent races of *L. maculans* is crucial for effective deployment of *R* gene-mediated resistance.

Methods: The release of ascospores in the air was monitored by using Burkard spore samplers at four different sites in the UK and the frequencies of avirulent *AvrLm1* and *AvrLm6* in the *L. maculans* ascospore populations were identified by qPCR. Winter oilseed rape field experiments were set up at four different sites in the UK. Single pycnidial isolates were obtained from leaf lesions on cultivar Drakkar from all the sites and pathogen identification was done by morphology on PDA and confirmed by species-specific PCR. Changes in the frequencies of avirulent *AvrLm1*, *AvrLm4*, *AvrLm6* or *AvrLm7* alleles in *L. maculans* populations at different sites in the UK were investigated by inoculation of conidial suspensions on the cotyledons of a differential set of cultivars.

Results: There were differences between the four sites in patterns of ascospore release and in dates of first major ascospore release. There were also differences between sites in the timing of release of ascospores with avirulent *AvrLm1* and *AvrLm6* alleles. *AvrLm6* alleles were detected more frequently from spore samples compared to *AvrLm1* alleles. All the isolates tested from different sites were avirulent against *Rlm7*. There were variations between sites in the frequencies of avirulent *AvrLm1* and *AvrLm4* alleles. None of the isolates from different sites were avirulent against *Rlm3* (*AvrLm3*) or *Rlm9* (*AvrLm9*).

Conclusions: The *AvrLm7* allele is predominant in the UK *L. maculans* populations suggesting that the corresponding *Rlm7* resistance gene is still effective. Virulent *avrLm3* and *avrLm9* alleles are predominant in the UK *L. maculans* populations suggesting that *Rlm3* and *Rlm9* resistance genes are no longer effective in the UK. There is a need to continue the monitoring the regional distribution of *L. maculans* populations in the UK for the effective deployment of *R* genes.

55. DECREASING THE RISK OF SEVERE PHOMA STEM CANKER CAUSED BY *LEPTOSPHAERIA BIGLOBOSA* ON WINTER OILSEED RAPE IN UK

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Introduction: Oilseed rape is the third most important arable crop in the UK. Phoma stem canker is a damaging disease of the crop that has caused £100M crop losses per growing season. This disease is caused by two fungal pathogens; *Leptosphaeria maculans* and *L. biglobosa*. *L. maculans* is generally considered more damaging, causing stem base cankers whereas *L. biglobosa* has been associated with the less damaging upper stem lesions. However, recent studies suggest that *L. biglobosa* can cause both stem base cankers and upper stem lesions leading to severe yield losses. Furthermore, *L. biglobosa* is less sensitive to some triazole fungicides than *L. maculans* and no resistance against *L. biglobosa* has been bred into cultivars. This work aimed to understand the importance of *L. biglobosa* in causing phoma stem canker and to improve control by targeting both *L. maculans* and *L. biglobosa* using fungicides.

Methods: To determine the prevalence of the two pathogens in the air, ascospore release was monitored from September 2015 to March 2016, using Burkard spore samplers set up at four sites in the UK. The spore tapes from the spore samplers were cut into two halves; one half was used for microscopic spore counting whereas the other half was used for DNA extraction and qPCR to determine the proportions of *Leptosphaeria* spp. in the air samples. To investigate the effects of fungicides on control of phoma stem canker, a field trial was set up in the 2015-2016 growing season at Boxworth UK. Six cultivars with different 'field' resistance to phoma stem canker pathogens were selected and sown in a randomised block design with three replicates. The cultivars were treated with two fungicides (Proline and Refinzar) and assessed for phoma leaf spotting and phoma stem canker.

Results: Data from the microscopic analysis of spore tapes showed that there were differences between the four sites in the timing of first major ascospore release and maximum ascospore release. Ascospores were released from September until February at all four sites. The qPCR analysis of spore tapes indicated that there was more *L. maculans* DNA than *L. biglobosa* detected at three sites whereas there was more *L. biglobosa* DNA than *L. maculans* DNA detected at one site. In the field experiment, both Proline and Refinzar did not reduce the severity of *L. maculans* and *L. biglobosa* phoma leaf spots but they reduced the severity of phoma stem canker on all cultivars. Both fungicides reduced the severity of phoma stem canker more on susceptible cultivars as compared to resistant cultivars.

Conclusions: There were differences between sites in the timing of first major ascospore release and maximum ascospore release. The timing of first major ascospore release can be used to guide the ideal timing of fungicide sprays. Both fungicides did not control phoma leaf spotting but they reduced phoma stem canker severity. These experimental data can help in choice of cultivars and effective fungicides in local areas**.**

56. FINE MAPPING OF A MAJOR GENE LOCUS FOR RESISTANCE AGAINST *PYRENOPEZIZA BRASSICAE* (LIGHT LEAF SPOT) IN *BRASSICA NAPUS* AND IDENTIFICATION OF CANDIDATE RESISTANCE GENES

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Introduction: Plant major gene-mediated resistance plays an important role in the management of disease threats to crops. Fine mapping and cloning of major resistance genes can provide insights into host-pathogen interactions to improve disease control strategies. This work on the *Brassica napus* – *Pyrenopeziza brassicae* pathosystem aims to provide improved understanding of the operation of major gene-mediated resistance against hemibiotrophic plant pathogens. Light leaf spot disease, caused by *Pyrenopeziza brassicae*, is currently the most damaging foliar disease on winter oilseed rape in the UK. *P. brassicae* is also able to infect and cause the disease on vegetable brassicas, including Brussels sprouts. There is a major gene locus mapped to the bottom end of the *B. napus* chromosome A01 *c*. 3.0 cM away from the closest flanking marker. With the novel genomic information and molecular marker data on *B. napus*, it is expected that improved marker resolution can be obtained, leading to a better understanding of the genetic basis of this resistance.

Methods: A flanking marker close to the resistance gene on chromosome A1 was PCR-amplified from the parental lines of a doubled haploid (DH) population segregating for resistance and the PCR product was sequenced. The physical location of this marker on chr. A1 in *B. napus* was identified by using marker sequence alignment to the *B. rapa* genome and the synteny between *B. rapa* & *B. napus* genomes.

Results and discussion: Currently, we have identified the physical location of the closest flanking marker to the resistance locus on *B. napus* chrA01. The chromosomal region between this locus and the telomere in *B. napus* spans *c.* 1.08Mbp. Single nucleotide polymorphism (SNP) marker information on the corresponding genomic region has been obtained and *c.* 400 candidate SNPs specific to chrA01 have been identified. Initially, 38 SNPs were selected for the development of KASP markers. These markers will be tested on the parental lines of the DH population that segregate for the major gene for resistance against *P. brassicae*. Polymorphic markers between parental lines will be analysed in the segregating population to improve the resolution of the genetic map. In parallel, we analyse three different *Brassica napus* genome sequences to identify gene content in this genomic region and gene predictions will be used to identify candidate resistance genes against *P. brassicae.*

57. IMPROVED MANAGEMENT OF LIGHT LEAF SPOT BY UNDERSTANDING THE STRUCTURE OF *PYRENOPEZIZA BRASSICAE* POPULATIONS

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Introduction: Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is currently the major disease problem in oilseed rape (*Brassica napus* L.) production in the UK and also affects vegetable brassicas such as cabbage, cauliflower and Brussels sprouts. The disease was considered a problem in Scotland and North England but has substantially increased importance in all parts of England over the last decade. Due to the polycyclic nature of the disease, the pathogen has the potential to adapt to an environment (McDonald & Linde, 2002). Effective control of light leaf spot to reduce yield and economic losses is difficult to achieve. Fungicide control of the disease in crops is difficult since fungicides must be applied when the pathogen is growing asymptomatically in plant tissues (Figueroa et al. 1994). Additionally, decreased sensitivity to azole fungicides has been reported (Carter et al. 2013). Exploiting plant resistance against the pathogen could help control the disease but current commercial cultivars show poor resistance. The project focuses on the determination of the population structure and the host range of *P. brassicae.*

Methods: Field assessments with 10 cultivars were done at five locations in England and one location in Scotland to discover possible shifts in pathogenicity towards specific cultivars. Furthermore, oilseed rape and vegetable brassicas were inoculated with *P. brassicae* to identify the host range and possible gene-for-gene interactions.

Results: Oilseed rape cultivars showed differences in susceptibility to *P. brassicae* at different locations. Cultivar differences were also recorded in *in planta* experiments. Pathogen populations of oilseed rape and Brussels sprouts were able to infect other vegetable brassicas.

Conclusions: The results suggest that different pathogen populations may be present at different locations. With increased information about pathogen populations regional advice for deployment of cultivars can be given to farmers for a more effective use of cultivar resistance. Areas with cultivation of diverse brassica crops may expect more severe disease epidemics of light leaf spots due to the ability of *P. brassicae* populations to cross-infect between vegetables and oilseed rape.

Acknowledgements: We are grateful for funding from AHDB Cereals & Oilseeds and AHDB Horticulture, Felix Thornley Cobbold Agricultural Trust, University of Hertfordhire and in-kind support from ADAS, Elsoms Seeds, Limagrain UK and Trialsforce. We are thankful to the Georg-August university of Goettingen as well as Elsoms Seeds for hosting *in planta* experiments.

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58. WHAT IS THE ORIGIN OF THE INITIAL INOCULUM FOR STRAWBERRY POWDERY MILDEW EPIDEMICS?

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Introduction : In the last 25 years, strawberry growing in the UK has been transformed, with yields going up whilst the number of hectares used has gone down. The UK now grows 80% of the strawberries we eat. This has been achieved by precision choice of varieties, the use of fertigation, and the use of fleece and mulch in the spring and use of polythene tunnels primarily during harvest. Everbearer strawberries are harvested from May to September so crops are covered by tunnels for up to 5 months. The conditions of temperature and humidity under the polythene tunnels are ideal for the development of strawberry powdery mildew. Controlling strawberry powdery mildew is a major problem for strawberry growers and the bigger the epidemic, the higher the cost of control. Growers tend not to see early disease development and then be taken by surprise when the epidemic builds up very suddenly. The question is often 'Where did the disease come from?'

Methods: Since 2011 we have monitored the overwintering of the fungus on strawberry plants in the ground for 3 winters and 4 summers. Also, all plants coming onto the farm for one year planting have been monitored when they arrive on the farm. Growers source these plants from the UK and continental Europe.

Results: Results from the propagators between 2011 and 2015 show that the amount of diseased plants range from 6.5% and 90% of leaflets sampled with strawberry powdery mildew. Propagators were situated in the UK, Netherlands and Spain. Chasmothecia survived throughout the winter and numbers of chasmothecia were reduced when autumn and spring fungicide sprays were used. These reduced the initial inoculum.

Conclusion: Plants from propagators are not guaranteed 'disease free'. Growers are advised to closely monitor early disease symptoms on plants, and to apply fungicides when necessary.

59. INVESTIGATING THE EFFECT OF WOUNDING ON THE *BRASSICA NAPUS* DEFENCE RESPONSE

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Introduction: Each year losses in UK oilseed rape production due to phoma stem canker cost *c.* £80 million. In an effort to control this fungal disease, farmers grow resistant cultivars. Cultivar resistance may be described as either quantitative or qualitative in nature. Quantitative resistance is generally controlled by several genes. In contrast, qualitative resistance is controlled by single, dominant major resistance (*R*) genes, which protect against specific pathogen races. Qualitative resistance has commonly been found to rapidly become ineffective because single *R* genes exert selection on the pathogen population. There is a need to study temperature-sensitivity of oilseed rape *R* genes against *Leptosphaeria maculans*. Cotyledon tests provide a key role in this area of study as they can be used to analyse host defence responses to the pathogen both by physiological observation and through gene expression. However, it has been suggested that a wound response caused by the wounding action involved in the inoculation of these cotyledons may interfere with the host defence response against the pathogen, thus skewing results.

Method: To determine whether the wound response impacts upon the host defence response, seedlings were inoculated by either wound or infiltration and subsequent gene expression was compared. Quantitative PCR was done to compare the expression of genes associated with wounding (lipoxygenase (*LOX*) and the plant defence response (*PR1)* between the two inoculation methods at various time points post inoculation.

60. UNDERSTANDING HOW GOVERNMENT IN THE REPUBLIC OF CYPRUS IS RESPONDING TO THE ISSUES OF WATER SCARCITY AND QUALITY

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Introduction: Water management is a significant problem in The Republic of Cyprus. The country is subject to a number of water resource problems stemming from limited precipitation inputs, the overuse of groundwater, as well as the spatial disparity of water supply and demand due to population growth, agriculture, tourism, and climate change. These issues serve to shape water management responses. Overall, the convergence of these issues has generated water resource problems, which necessitate particular problem-solving responses by government that are targeted at securing the provision of water services and sustaining socio-economic development. Attempts to conceptually understand how governments tackle the problems associated with the provision of water is a neglected area in the literature focused on water management and policy, particularly in the context of Cyprus and from perspectives of political economy. Therefore, in order to overcome this lack of understanding, the research aims to explore how water problems in Cyprus can be understood from the perspectives of administrative rationalism, democratic pragmatism, and economic rationalism.

Methods: The research adopts a qualitative approach to data collection, and utilises a semi-structured interview to understand the views, roles, and experiences of key actors in the decision-making process. A case study approach is also used to provide an appropriate context and facilitate detailed analysis of the problem-solving rationalities. In essence, Cyprus offers a unique and relevant setting based on a range of key factors, including; limited previous research considering water policy, problem-solving responses, and decision-making in a Cypriot context; a tangible water management problem; a complex socio-political setting that involves the management of shared resources; as well as the status of Cyprus as a liberal democratic society and an EU Member State.

Results: A range of findings and general themes have been distinguished as a result of thematic and content analysis of data collected from the interviews. The themes identified include; the importance, role, and influence of politics in decision-making; the evidence of sequential rationalities; the understanding of actor roles, behaviour, and the concept of self interest; the role and influence of supranational governance; as well as the importance of external factors such as culture.

Conclusion: The findings show that Dryzek's rationalities are both valid and limited in their conceptual understanding of problem-solving. For instance, certain aspects are shown to be justifiable, such as the basis of administrative rationalism emerging as a first problem-solving response and the idea that some civil servants can and do try to act in the public interest. However, drawbacks of the rationalities are also apparent including; the limited analysis of politics and its influence in reality through political culture and behaviour; a poor representation of supranational governance and the influence it has on national government; as well as an inadequate evaluation of the influence of external factors such as inherent culture and economic status.