S02
Hemopexin counteracts systolic dysfunction induced by heme overload
Can-Martin Sagi1; Nikola Revi1; Stefan Wagner1; Lucia De Franceschi1; Francesca Vechi1; James Cimino2; Sara Petrillo3; Lorenzo Sienega; Fiorella Atruda4; Lars S. Måger5; Emilke Hirsch1; Alessandra Ghigo6; Emanuela Tosiello11
1Department of Cardiology, Universitàtsklinikum Regensburg, Germany; 2Department of Cardiology, Universitàtsklinikum Regensburg, Germany; 3Department of Neurology, Universitàtsklinikum Regensburg, Germany; 4Department of Cardiology, Universitàtsklinikum Regensburg, Germany; 5Department of Biotechnology and Health sciences, University of Turin, Italy; 6Department of Biotechnology and Health sciences, University of Turin, Italy; 7Department of Biotechnology and Health sciences, University of Turin, Italy; 8Department of Biotechnology and Health sciences, University of Turin, Italy; 9Department of Biotechnology and Health sciences, University of Turin, Italy; 10Department of Biotechnology and Health sciences, University of Turin, Italy; 11Department of Biotechnology and Health sciences, University of Turin, Italy

Purpose: Hemopexin is the plasma protein with the highest binding affinity for heme. Recently, it was demonstrated that hemopexin may be used as a therapeutic tool to prevent endothelial damage in animal models of hemolytic diseases. It is well known that during hemolytic diseases, not only the vessels, but also the heart is one of the most organs affected by iron and heme damage. The purpose of this work is to understand if Hemopexin could have a protective role in the cardiac system, under basal and pathological conditions.

Methods: To investigate the mechanism of hemopexin (Hx)-mediated protection, we analyzed Hx+/− and Hx−/+ mice under basal conditions and β-thalassemic mice, as pathological model of cardiac heme toxicity. Moreover, we performed experiments on primary cardiomyocytes treated with heme in the presence or absence of hemopexin.

Results: We found that the heart of Hx+/− mice accumulated heme. Heme accumulation resulted in the up-regulation of Heme Oxygenase (H O)-1 and Ferroportin expression and in the down-regulation of Transferrin Receptor (TR)1 and Divalent Metal Transporter 1 (DMT1), and it was associated with increased oxidative stress. These data were confirmed on neonatal cardiomyocytes. Heme loading in the heart of Hx+/− mice led to oxidation of Calcium/calmodulin-dependent protein kinase II (CaMKII). We also found modifications in the oxidation and phosphorylation of Ryano
dine receptor 2 (RYR2) at the serine 2814 and in Phospholamban (PLB) at threonine 17, suggesting a role of CaMKII in these modifications.

Conclusion: We found that hemopexin could have a protective role in the cardiac system, under basal and pathological conditions. Further research is needed to understand the molecular mechanisms of heme uptake and cardiac remodeling induced by heme overload.
Conclusion: This work demonstrates that defibrillation energy can be reduced by applying tailored stimulation patterns, opening translational perspectives for improving current anti-arrhythmic therapies.

505 Tachypacing-induced heart failure: a metabolomic investigation

Amy Watkins¹; Stephanie Church²; Paul Begley³; David Esiner⁴; Andrew Trafford⁵; Garth Cooper⁶
¹University of Manchester; ²Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; ³Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK; ⁴Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; ⁵Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK; ⁶Institute for Cardiovascular Sciences, University of Manchester, Manchester, UK; ⁷School of Biological Sciences, Faculty of Science and Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, New Zealand; ⁸Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; ⁹Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; ¹⁰Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK; ¹¹Department of Pharmacology, Medical Sciences Division, University of Oxford, Oxford, UK

Background: Heart failure (HF) refers to a complex disease state whereby cardiac function has deteriorated to such a degree that the heart is unable to cope with the demands of the body. Despite the economic and social burden of HF little is understood about the metabolic profile underlying the disease.

Purpose: We applied untargeted GC-MS and LC-MS to investigate the metabolic profile of cardiac left ventricle (LV) tissue during the induction and reversal of HF using an ovine tachypacing model.

Methods: HF was induced in female sheep by right ventricular tachypacing at 210 bpm until symtomatic end-stage HF. A second cohort of animals was allowed to recover at the point of end-stage HF for five weeks by ceasing tachypacing. LV free wall was harvested from control (n = 7) and their wild type (WT) littermates mice (n = 7) were used. LV function was measured using inserting the catheter tip retrograde into LV. mRNA levels of cytokines and chemokines (IL-6, IL-1β, MCP-1, MIP2 and KC), MMP12 was measured by RT-qPCR.

Results: HF mice showed severe arthritis such as paw swelling in association with smaller body weight and heart weight in comparison to WT littermates (P < 0.01). LV systolic pressure and the rate of LV pressure rise was decreased in HF mice (P < 0.01). In comparison with WT littermates mRNA expression of MCP-1 and KC were increased in HF mice (P < 0.01).

Conclusion: MMP12 mice showed attenuated ERBb expression, irrespective of RA.

Acknowledgements: The University of Manchester; ²Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; ³Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; ⁴Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK; ⁵Department of Pharmacology, Medical Sciences Division, University of Oxford, Oxford, UK

Introduction & purpose: There is a circadian rhythm in heart rate (HR), which slows at night. We hypothesised that it is the result of an intrinsic circadian clock in the pacemaker, the sinus node.

Results: In conscious C57BL/6J mice maintained during a 12 h light-12 h dark cycle, there was a circadian rhythm in HR with end-stage HF for If) was measured at the mRNA level by qPCR; in addition, expression of HCN4 protein was measured by Western blot. Clock genes (e.g. CLOCK and BMAL1) were expressed and varied in a circadian manner (P < 0.05). HCN4 mRNA was 89% higher at ZT0 compared to ZT12 whereas HCN4 protein was 49% lower (P < 0.05).

Conclusions: Overall, these data may suggest a pathological metabolic disturbance is occurring during pacing-induced HF and its reversal, with specific focus on altered energy substrate utilisation and lipid metabolism.

506 Characterization of early left ventricle dysfunction in a relevant experimental model for human rheumatoid arthritis

Silvia Hayen¹; Milat Inci²; Birgit Niederreiter³; Tetyana Shvets⁴; Kurt Redlich⁵; Attila Kiss⁶
¹Department of Internal Medicine III, Medical University of Vienna, Austria; ²Maastricht University, Maastricht; ³Department of Internal Medicine III, Medical University of Vienna, Austria; ⁴Department of Internal Medicine III, Medical University of Vienna, Austria; ⁵Medical University of Vienna, Medical University of Vienna, Austria; ⁶University of Manchester; ⁷The University of Manchester, UK; ⁸The University of Manchester, UK; ⁹The University of Manchester, UK; ¹⁰The University of Manchester, UK; ¹¹Medical University of Vienna, Department for Biomedical Research, Austria

Background: Rheumatoid arthritis (RA) is associated with left ventricle (LV) hemodynamic dysfunction which may be due to the upregulation of the circulatory levels of cytokines and chemokines. However, their expression and contribution to LV dysfunction in RA heart are unknown. The activation of neurotrophic (NRG1) and/or receptor Tyrosine Kinase 2 and 4 (Erbb2 and Erbb4) pathway is considered to protect the myocardium and the impairment of this pathway, ultimately contributes to the development of heart failure.

Aim: Characterize LV function and determine the expression of inflammatory cytokines, chemokines, NRG1/Erbb in hearts of a TNF-driven inflammatory, erosion arthritis model.

Methods: Anae sthetized and intubated male and female 14-15 weeks old human TNF-alpha transgenic (hTNFg; n = 7) and their wild type (WT) littermates mice (n = 7) were used. LV function was evaluated by inserting the catheter tip retrograde into LV. mRNA levels of cytokines and chemokines (IL-6, IL-1β, MCP-1, MIP2 and KC), MMP12 was measured by RT-qPCR.

Results: hTNFg mice showed severe arthritis such as paw swelling in association with smaller body weight and heart weight in comparison to WT littermates (P < 0.01). LV systolic pressure and the rate of LV pressure rise was decreased in hTNFg mice (P < 0.01). In comparison with WT littermates mRNA expression of MCP-1 and KC were increased in hTNFg mice (P < 0.01).

Conclusion: hTNFg mice have showed impaired LV systolic function in association with the upregulation of MCP-1 and KC. NRG1 expression increased in both gender which might be a compensatory mechanism downregulation of Erbb2 was observed in female mice, irrespective of RA. These results may represent a potential novel therapeutic point to improve LV function and reduce risk of cardiovascular disease in RA.

507 Circadian rhythm in heart rate is due to an intrinsic circadian clock in the sinus node

Yanwen Wang¹; Alicia D’Souza²; Anne Bert Johnsen³; Servé Olieslagers⁴; Annalisa Bucchi⁵; Paula Da Costa Martins⁶; Dario DiFrancesco⁷; Helina Dobrynicki⁸; Hugh D. Piggins⁹; Mark R. Boyett¹⁰
¹The University of Manchester; ²The University of Manchester, UK; ³Norwegian University of Science and Technology, Norway; ⁴Maastricht University, Maastricht; ⁵The University of Milan, Italy; ⁶Maastricht University, Maastricht; ⁷The University of Milan, Italy; ⁸The University of Manchester, UK; ⁹The University of Manchester, UK; ¹⁰The University of Manchester, UK

Introduction & purpose: There is a circadian rhythm in heart rate (HR), which slows at night. We hypothesised that it is the result of an intrinsic circadian clock in the pacemaker, the sinus node.

Methods: In conscious c57Bl/6 mice maintained during a 12 h light-12 h dark cycle, there was a circadian rhythm in HR with end-stage HF for If) was measured at the mRNA level by qPCR; in addition, expression of HCN4 protein was measured by Western blot. Clock genes (e.g. CLOCK and BMAL1) were expressed and varied in a circadian manner (P < 0.05). HCN4 mRNA was 89% higher at ZT0 compared to ZT12 whereas HCN4 protein was 49% lower (P < 0.05).

Conclusions: Overall, these data may suggest a pathological metabolic disturbance is occurring during pacing-induced HF and its reversal, with specific focus on altered energy substrate utilisation and lipid metabolism.