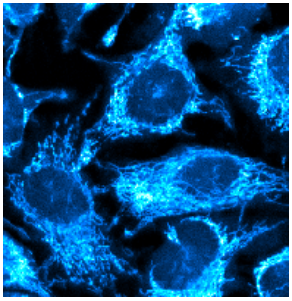
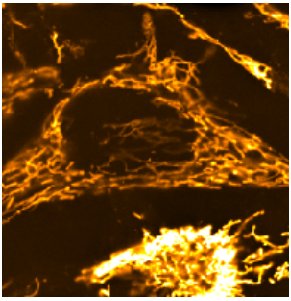
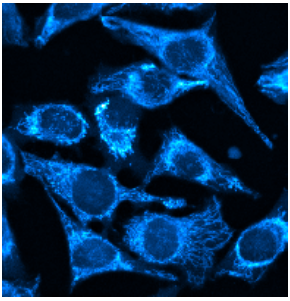
Mitochondria:
Form and function

Thursday 14 – Friday 15 September 2017

Mary Ward House, London, UK

Scientific programme organisers

- Sean Davidson, University College London, UK
- Michael Duchen, University College London, UK
- Beatrice Filippi, University of Leeds, UK
- Andrew Philp, University of Birmingham, UK

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Mitochondria: Form and function

Joint meeting of the British Pharmacological Society, the Biochemical Socity & The Physiological Society

Organised by Sean Davidson, University College London, UK, Michael Duchen, University College London, UK, Beatrice Filippi, University of Leeds, UK and Andrew Philp, University of Birmingham, UK

Thursday 14 - Friday 15 September 2017

Mary Ward House Conference and Exhibition Centre
5 - 7 Tavistock Place, London WC1H 9SN, UK

Thursday 14 September

8.50 **Welcome**

9.00 PLENARY LECTURE
A bioenergetic basis for the three domains of life
PL01 Nick Lane, University College London, UK

CALCIUM AND MITOCHONDRIA

Chair: Sean Davidson, University College London, UK

10.00 **Calcium and beyond: cation channels in the inner mitochondrial membrane**
SA01 Diego De Stefani, University of Padova, Italy

10.30 **Mitochondrial bioenergetic signaling drives myofibroblast differentiation**
SA02 John Elrod, Temple University, USA

11.00 **Break**

11.30 **The structure of the mitochondrial permeability transition pore and design of novel inhibitors**
SA03 Valentina Giorgio, University of Padova, Italy

12.00 **Ca^{2+}-induced mitochondrial ROS regulate the early cell cycle in Xenopus embryos**
C01 Enrique Amaya, University of Manchester, UK

12.15 **GBA1 deficiency sensitises neurons to glutamate toxicity and causes calcium dyshomeostasis**
C02 Nicoletta Plotegher, University College London, UK

12.30 **Posters & lunch**

FISSION AND FUSION

Chair: Gyorgy Szabadkai, University College London, UK

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SA04 Josef Kittler, University College London, UK

14.30 **Mitochondrial fission and stress**
SA14 Alexander van der Bliek, UCLA, USA

15.00 **Mitochondrial fusion dynamics in the skeletal muscle as a target of fibrosis**
SA05 Verónica Eisner, Catholic University of Chile, Chile

15.30 **Break**

16.00 **The role of mitochondrial fusion proteins on liver metabolism**
SA06 Antonio Zorzano, IRB Barcelona, Spain

16.30 **Mitochondria fission in the dorsal vagal complex induces insulin resistance**
SA07 Beatrice Filippi, University of Leeds, UK

16.45 **Amyloid-β oligomers or hyperglycaemia each reduce mitochondrial motility in mature hippocampal cells**
C03 Susan Chalmers, University of Strathclyde, UK

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17.00 - 18.00 Odd poster numbers
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Friday 15 September

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Chair: Hélène Plun-Favreau, University College London, UK

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SA08 Ian Ganley, University of Dundee, UK

10.00 **Autophagy-mitochondria axis in health in disease**
SA09 Viktor Korolchuk, Newcastle University, UK

10.30 **Mitochondrial remodeling: Rearranging, recycling, and reprogramming**
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11.00 **Assessing mitochondrial structure and function in C. elegans muscle**
C04 Amelia Pollard, University of Nottingham, UK

11.15 **Components of the innate immune system evoke mitochondrial membrane hyperpolarisation, increases in mitochondrial reactive oxygen species via Nox and induce cell death in the renal medulla**
C05 Kirsti Taylor, University of Kent, UK

11.30 **Break**

12.00 **Krebs cycle intermediates drive abnormal metabolic signalling in response to hypoxia in the diabetic heart**
C06 Lisa Heather, University of Oxford, UK

12.15 Low levels of the A3243G mitochondrial DNA mutation in human induced pluripotent stem cell-cardiomyocytes do not cause functional or metabolic disturbances but increase with further passaging
C07 Carolina Pinto Ricardo, Imperial College London, UK

12.30 Posters & lunch

13.30 Physiology and pathology of alpha-synuclein
C08 Marthe Ludtmann, University College London, UK

13.45 Preventing Glyoxalase-1 (Glo-I) downregulation attenuates mitochondria dysfunction in hearts of diabetic rats
C09 Jaipaul Singh, University of Central Lancashire, UK

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Chair: Andy Philp, University of Birmingham, UK

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SA11 Robert Balaban, National Institutes of Health, USA

14.30 Exercise and skeletal muscle mitochondria
SA12 Francesca Amati, University of Lausanne, Switzerland

15.00 Break

15.30 The effect of maternal diet & obesity in pregnancy on mitochondrial function in the offspring
SA13 Carrie McCurdy, University of Oregon, USA

Friday 15 September

16.00 Evaluation of a novel mitochondria-targeted peptide based H2S donor compound (RTP-10) in hyperglycaemia-induced microvascular endothelial cell dysfunction
C10 Roberta Torregrossa, University of Exeter, UK

16.15 Type 2 diabetic mitochondria overcome fatty acyl CoA inhibition, but have reduced respiration
C11 Matt Kerr, University of Oxford, UK

16.30 PLENARY LECTURE
Targeting mitochondria for therapy
PL02 Rong Tian, University of Washington, USA

17.30 End of Topic Meeting

C02

GBA1 deficiency sensitises neurons to glutamate toxicity and causes calcium dyshomeostasis

N. Plotegher[1], D. Perocheau[3], G. Massaro[4], A. Rahim[2], S. Waddington[3] and M. Duchen[1]

[1]Department of Cell and Developmental Biology, University College London, London, UK, [2]School of Pharmacy, University College London, London, UK, [3]Institute of Women's Health, University College London, London, UK and [4]School of Pharmacy, University College London, London, UK

C03

Amyloid-β oligomers or hyperglycaemia each reduce mitochondrial motility in mature hippocampal cells

S. Chalmers[1], R. Rooney[1], S. Albazi[1] and C.D. Saunter[2]

[1]SIPBS, University of Strathclyde, Glasgow, UK and [2]Physics, Durham University, Durham, UK

C04

Assessing mitochondrial structure and function in C. elegans muscle

A. Pollard[1], C. Gaffney[2], J. Hewitt[3], S. Vanapalli[3], D. Constantin-Teodosiu[4], P. Greenhaff[4], T. Etheridge[2] and N.J. Szewczyk[1]

[1]MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Derby, UK, [2]School of Sport & Health Sciences, University of Exeter, Exeter, UK, [3]Department of Chemical Engineering, Texas Tech University, Lubbock, TX, USA and [4]MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Nottingham, UK

C05

Components of the innate immune system evoke mitochondrial membrane hyperpolarisation, increases in mitochondrial reactive oxygen species via Nox and induce cell death in the renal medulla

K.D. Taylor, S. Wildman and C.M. Peppiatt-Wildman

Medway School of Pharmacy, University of Kent, Kent, UK

C07

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C. Pinto Ricardo[1], N. Hellen[2], G. Foldes[1], T. Kodagoda[1], C. Terracciano[1], M. Duchen[3] and S. Harding[1]

[1]Imperial College London, London, UK, [2]Imperial College London, London, UK and [3]University College London, London, UK

C08

Physiology and pathology of alpha-synuclein

M. Ludtmann, P. Angelova, M. Choi, S. Gandhi and A. Abramov

Institute of Neurology, London, UK

C09

Preventing Glyoxalase-1 (Glo-I) downregulation attenuates mitochondria dysfunction in hearts of diabetic rats

J. Singh[1], F.M. Alomar[2,3] and K.R. Bidasee[4]

[1]University of Central Lancashire, Preston, UK, [2]University of Nebraska Medical Center, Omaha, NE, USA, [3]University of Dammam, Dammam, Saudi Arabia and [4]Nebraska Redox Biology Center, Lincoln, NE, USA

C10

Evaluation of a novel mitochondria-targeted peptide based H2S donor compound (RTP-10) in hyperglycaemia-induced microvascular endothelial cell dysfunction

R. Torregrossa[1], A. Waters[1], D. Gero[2], A. Perry[1], S. Webb[3], C. Rush[3], M. Wood[1] and M. Whiteman[1]

[1]University of Exeter, Exeter, UK, [2]Institute of Pathophysiology, Faculty of Medicine, Semmelweis University, Semmelweis, Hungary and [3]ISCA Biochemicals, Exeter, Exeter, UK

C11

Type 2 diabetic mitochondria overcome fatty acyl CoA inhibition, but have reduced respiration

M. Kerr, S. Rohling, M. Sousa Fialho, C. Lopez, D. Tyler and L. Heather
Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

PC01

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P. Coats and Z. Al-Sulti
SIPBS, University of Strathclyde, Glasgow, UK

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C. Varricchio
School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

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E. Cocks, K. White and M. Taggart
Newcastle University, Newcastle Upon Tyne, UK

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M. Carisi, D.J. Rees, A.H. Morgan and J.S. Davies
Medical School, Swansea University, Swansea, UK

Poster communications

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M. O'Reilly[1], N. Kirkwood[1], M. Derudas[2], E. Kenyon[1], G. Richardson[1] and C. Kros[1]

[1]Sussex Neuroscience, University of Sussex, Brighton, UK and [2]Sussex Drug Discovery Centre, University of Sussex, Brighton, UK

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A. Kirby[1], M. Yang[1], M. Radeke[2] and P. Turowski[1]

[1]Institute of Ophthalmology, University College London, London, UK and [2]Neuroscience Research Institute, UCSB, Santa Barbara, CA, USA

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A.I. Tarasov, J. Knudsen, J. Adam, M. Chibalina and P. Rorsman

OCDEM, University of Oxford, Oxford, UK

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D. Schiza[1], F. Peters[1], M. Clark[1], M. Duchen[2] and K. Smith[1]

[1]Neuroinflammation, UCL, London, UK and [2]Cell and Developmental Biology, University College London, London, UK

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R. Wüst[1], N. Held[1], E. van Deel[2], D. Kuster[2] and R. Houtkooper[1]

[1]Amsterdam Medical Center, Amsterdam, Netherlands and [2]VU Medical Center, Amsterdam, Netherlands

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T. Etheridge[1], C. Gaffney[1], N.J. Szewczyk[2], R. Torregrossa[1], M. Wood[1] and M. Whiteman[1]
[1]University of Exeter, Exeter, UK and [2]University of Nottingham, Nottingham, UK

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M. Penke[2], A. Garten[1],[2], A. Barnikol-Oettler[2], T. Gorski[2], G. Lavery[1] and W. Kiess[2]
[1]University of Birmingham, Birmingham, UK and [2]Hospital for Child and Adolescent Medicine, Leipzig University, Leipzig, Germany

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S.P. Ashcroft[1], J. Bass[2], N.J. Szewczyk[2], P. Atherton[2] and A. Philp[1]
[1]Sport, Exercise & Rehabilitation Sciences, University of Birmingham, Birmingham, UK and [2]School of Medicine, University of Nottingham, Derby, UK

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S. Bashir, S. Ayoub and W. Morgan
School of Health, Sport & Bioscience, University Of East London, London, UK

PC14

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J. Iglesias-Gonzalez, C. Thomson, S. Ishibashi and E. Amaya
Cell-Matrix Research Dvision, University of Manchester, UK

Poster communications

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E. Adeghate[1], C. Howarth[1], K.R. Bidasee[3] and J. Singh[2]
[1]College of Medicine, United Arab Emirates University, United Arab Emirates University, Al Ain, United Arab Emirates, [2]University of Central Lancashire, Preston, UK and [3]University of Nebraska Medical Center, Omaha, NE, USA

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B. Patel and B.M. Filippi
Faculty of Biological Sciences, University of Leeds, Leeds, UK

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Mai Alzaydi, Vahitha Abdul-Salam and Beata Wojciak-Stothard
Imperial College London, UK

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Robert Bentham
University College London, UK

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E. Palma[1, 2], A. Riva[1, 2], V. Iansante[3], S. Mudan[4], N. Manyakin[4], D. Morrison[4], C. Moreno[5, 6], D. Degré[5, 6], E. Trepo[5, 6], P. Sancho-Bru[7, 8], J. Altamirano[7, 9], J. Caballeria[7, 8], R. Miquel[10], G. Odena[11], R. Bataller[11], R. Williams[1, 2], S. Chokshi[1, 2]

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All refreshments are included in your registration at times detailed in the programme.

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Thursday 14 September from 19.30
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The Physiological Society

PL01
A bioenergetic basis for the three domains of life

Nick Lane
Department of Genetics, Evolution and Environment, University College London, UK

The deep differences between the three domains of life are puzzling and unexplained. Bacteria and archaea are almost indistinguishable in their size and morphology, but are strikingly distinct in their biochemical and metabolic properties, differing, for example, in the genes responsible for glycolysis, cell membrane, cell wall, and even DNA replication. In contrast, eukaryotes are chimeric, with most of their informational genes deriving from archaea, and genes for intermediary metabolism, as well as membranes, deriving from bacteria. Eukaryotes apparently arose just once in 4 billion years of evolution, around 2 billion years ago. Genetic divergence alone cannot explain this evolutionary trajectory, as bacteria and archaea evolved nothing resembling the morphological complexity of eukaryotes, despite virtually unlimited exploration of genetic space. I will argue that the differences between bacteria, archaea and eukaryotes are not attributable to selection (or neutral evolution) operating on populations of cells, but rather to the structural constraints imposed by membrane bioenergetics. The divergence of bacteria and archaea could have arisen from the dependence of LUCA, the last universal common ancestor, on geologically sustained proton gradients in alkaline hydrothermal vents at the origin of life. The constraints imposed by membrane bioenergetics then prevented both prokaryotic groups from becoming larger and more complex over 4 billion years of evolution. These constraints were relaxed, at the origin of eukaryotes, by a singular endosymbiosis between an archaeal host cell (probably related to the Lokiarchaeota), and a proteobacterium, the ancestor of mitochondria. This rare endosymbiosis restructured the topology of eukaryotic genomes in relation bioenergetic membranes, with the

loss of mitochondrial genes enabling the accumulation of genes and gene families in the nucleus. In effect, eukaryotes have multi-bacterial power without the huge genomic overheads of copying thousands of full bacterial genomes. This extreme genomic asymmetry ultimately gave eukaryotes 3-5 orders of magnitude more energy availability per nuclear gene. The acquisition of mitochondria enabled a 15,000-fold increase in maximal genome size, encompassing around a 5-10-fold expansion in the number of protein-coding genes, coupled with a 1000-10,000-fold expansion in gene expression. Many basal eukaryotic traits, such as sex, probably evolved in the context of this singular endosymbiosis between two prokaryotes.

SA14
Mitochondrial fission and stress

A.M. Van Der Bliek, Biological Chemistry, David Geffen School of Medicine at UCLA, Los Angeles, CA USA

Functional studies of the mitochondrial outer membrane protein Fis1 uncovered a novel regulatory pathway with unexpected effects on mitophagy. Fis1 was initially thought to be the main recruitment factor for Drp1 on the surface of mitochondria, similar to its role in yeast, but Fis1 -/- cells have little or no fission defects. We discovered that Fis1 is still part of the mitochondrial fission complex in mammalian cells, but it most strongly affects downstream events, helping to guide the selective elimination of damaged mitochondria during mitophagy. Mutations in Fis1 disrupt downstream degradation events during mitophagy, leading to an accumulation of large LC3 aggregates. Our collaborators in the Youle lab (NIH) then showed that LC3 aggregates resulted from loss of Fis1 binding to the Rab7 GAP TBC1D15, which normally inhibits Rab7 activity and associates with the isolation membrane through interactions with LC3/GABARAP family members. We concluded that Fis1 and TBC1D15 control autophagic encapsulation of mitochondria by regulating Rab7 activity at the interface between mitochondria and isolation membranes. Rab7

indirectly promotes interactions between UVRAG (a core component of the VPS34-autophagy complex) and Endophilin B1 (Bif-1) at the ER, possibly explaining why Fis1 mutants overproduce autophagy membranes. Endophilin B is a BAR domain and SH3 domain containing protein, closely related to Endophilin A, which interacts with Dnm2 (Dynamin 2) at the plasma membrane during clathrin-independent endocytosis. To determine whether Dnm2 and Endophilin B1 similarly contribute to autophagy, we investigated the localization of these proteins and the effects of gene deletions on autophagy. We observed significant increases in co-localization of these proteins at the ER when autophagy is induced. However, these proteins have very different effects on the progression of autophagy. Endophilin B gene knockout inhibits autophagy, but Dnm2 knockout does not. Instead, Dnm2 -/- cells have more tightly woven ER networks and they show significant delays in ER fragmentation induced by calcium ionophores. These results suggest that Dnm2 affects ER scission. Further studies are needed to understand why Dnm2 and Endophilin B1 have such different effects at the ER even though they clearly interact during autophagy. We can, nevertheless, already conclude that Dnm2 and Endophilin B1 display novel interactions at the ER membrane where they control ER scission and autophagy, respectively.

PL02

Targeting mitochondria for therapy

R. Tian

University of Washington, Seattle, WA, USA

Mitochondrial dysfunction is a recognized mechanism in the pathogenesis of a number of chronic diseases including neurodegeneration, diabetes and cardiovascular diseases. However, there has been very limited success in drug development for mitochondrial dysfunction. Novel therapeutic targets are urgently needed. In a mouse model of mitochondrial Complex-I deficiency due to deletion of *Ndufs4* we found that decreased NADH oxidation in mitochondria led to lower NAD/NADH ratio and reduced NAD-dependent protein deacetylation. The resultant hyperacetylation of mitochondrial proteins rendered the heart highly sensitive to additional stress. Elevation of NAD^+ level by pharmacological or genetic strategies in mouse models of mitochondrial dysfunction could normalize the protein acetylation and restore the stress response in the heart. Furthermore, we have identified a number of mechanisms by which NAD^+-sensitive protein acetylation contributes to the hypersensitivity to stress. Supplementation of nicotinamide mononucleotide (NMN), a NAD^+ precursor, in a mouse model of Leigh syndrome, reduced blood lactate level and significantly extended lifespan. NMN also restored the NAD/NADH ratio in the heart with pathological hypertrophy and blunted the course of heart failure. The safety and tolerability of increasing intracellular NAD^+ level by nicotinamide riboside (NR) is now being investigated in heart failure patients. Collectively, these observations identify NAD(H) level and multiple NAD^+-sensitive mechanisms as viable therapeutic targets for mitochondrial dysfunction.

I confirm that the above ethical and content criteria have been met and understand that The Society reserves the right to reject the abstract should it not conform to the above guidelines.

SA01

Calcium and beyond: cation channels in the inner mitochondrial membrane

D. De Stefani

Department of Biomedical Sciences, University of Padova, Padova, Italy

The electron flow through the mitochondrial electron transport chain is coupled to proton pumping out of the organelle matrix, thus generating a large membrane potential. The potential energy is stored as capacitance and used for different activities, including ATP synthesis, heat production and cation transport, essentially of Ca^{2+} and K^+. Among these functions, the latter has been historically poorly

appreciated, mainly because of the lack of molecular information on mitochondrial channels mediating Ca^{2+} and K^+ entry.

In the case of Ca^{2+}, the deadlock was suddenly broken few years ago thanks to the identification of the key component of mitochondrial Ca2+ uptake machinery, the Mitochondrial Calcium Uniporter (MCU). Two features of mitochondrial calcium signaling have been known for a long time: i) mitochondrial Ca^{2+} uptake widely varies among cells and tissues, and ii) channel opening relies on the extramitochondrial Ca^{2+} concentration, with low activity at resting [Ca^{2+}] and a steep activation as soon as cytoplasmic [Ca^{2+}] rises. This sigmoidal relationship prevents on one hand mitochondrial Ca^{2+} overload and ion vicious cycling in resting cells, and on the other hand it concurrently ensures a prompt response to cellular stimulation that leads to an increase in energy production. This complexity requires a specialized and highly dynamic molecular machinery, with several primary components that can be variably gathered together in order to match cellular energy demands and protect from death stimuli. In line with this, MCU is now recognized to be part of a macromolecular structure known as the MCU complex that can include at least MCUb, EMRE, MICU1 and its isoforms. The ongoing elucidation of the identity and the genuine function of the MCU complex components is now providing the molecular understanding of the biophysical properties of mitochondrial Ca^{2+} uptake. Here I will first review our current understanding of the MCU complex and then present data on a novel regulator of organelle Ca^{2+} handling named MICU3, an EF-hand containing protein localized in the mitochondrial inter membrane space and preferentially expressed in neurons. I'll show that MICU3 forms a disulfide bond-mediated dimer with MICU1, but not with MICU2, and it acts as a highly-potent enhancer of MCU-dependent mitochondrial calcium uptake. Accordingly, silencing of MICU3 in primary cortical neurons significantly decreases mitochondrial calcium uptake. Surprisingly, downregulation of MICU3 also suppresses cytosolic calcium spiking induced by activation of synaptic NMDA receptors, thus suggesting that mitochondria actively participate in the decoding of synaptic activity.

In the case of K^+, the situation is more enigmatic. Seven different K^+ channels have been described in the IMM, but their molecular identity is still debated, especially in the case of ATP-sensitive K^+ channels. K_{ATP} are indeed widely distributed ion channels acting as sensors of cellular metabolism. They are present in the plasma membrane (pmK_{ATP}), where they couple cell excitability with energy availability. They are also reported to be located at the level of intracellular membranes, in particular in mitochondria, but in this context even their own existence is still a matter of debate. Here, I will describe a mitochondria localized protein complex that mediates ATP-sensitive potassium currents, the so-called $mitoK_{ATP}$. Similarly to its plasma membrane counterpart, the $mitoK_{ATP}$ is composed by a pore-forming subunit, named mitoK, together with an ATP-sensitive subunit, named mitoSUR. In vitro reconstitution of mitoK and mitoSUR recapitulates the main electrophysiological properties and pharmacological profile of the $mitoK_{ATP}$. This channel is normally closed at physiological ATP concentrations, thus preventing cations to enter the mito-

chondrial matrix and the consequent dissipation of mitochondrial membrane potential ($\Delta\Psi_m$). The overexpression of the channel forming subunit alone (mitoK) causes loss of $\Delta\Psi_m$, decrease of mitochondrial Ca^{2+} uptake, organelle fragmentation and disruption of cristae, in line with an increased cation permeability of the inner mitochondrial membrane. However, the concomitant overexpression of the mitoSUR subunit restores the correct channel gating and rescues organelle dysfunction. Conversely, mitoK ablation causes mitochondrial dysfunction characterized by instability of mitochondrial membrane potential accompanied by a decrease of oxidative performance. Overall, our data suggest that the mitoK$_{ATP}$ is a novel player in the regulation of mitochondrial physiology with a potential impact on many pathological processes such as ischemia-reperfusion injury and ageing.

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SA02

Mitochondrial bioenergetic signaling drives myofibroblast differentiation

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When the heart is injured quiescent fibroblasts differentiate into contractile, synthetic myofibroblasts. Initially fibrosis is reparative, but when chronic it becomes maladaptive and contributes to heart failure progression. Cytosolic Ca^{2+} ($_cCa^{2+}$) signaling is reported to be necessary for myofibroblast transdifferentiation, yet the role of mitochondrial Ca^{2+} ($_mCa^{2+}$) exchange has not been explored. $_cCa^{2+}$ signaling is rapidly integrated into the mitochondrial matrix via the $_mCa^{2+}$ uniporter channel (MCUc). To examine the contribution of $_mCa^{2+}$ in cardiac fibrosis, we generated conditional, fibroblast-specific *Mcu* knockout mice (KO) to ablate $_mCa^{2+}$ uptake. KO and control mice were subjected to myocardial infarction and cardiac function was examined by echocardiography. Loss of $_mCa^{2+}$ uptake worsened left ventricular function and increased fibrosis. To examine the cellular mechanisms responsible for the increased fibrosis we isolated mouse embryonic fibroblasts (MEFs) from *Mcu*$^{fl/fl}$ mice and deleted *Mcu* with Cre-adenovirus. When challenged with pro-fibrotic ligands (TGF-β and AngII), *Mcu*$^{-/-}$ MEFs exhibited decreased $_mCa^{2+}$ uptake and enhanced $_cCa^{2+}$ transient amplitude. Loss of *Mcu* promoted myofibroblast differentiation, including increased α-SMA expression and enhanced contractile function. *Mcu*$^{-/-}$ MEFs were more glycolytic with increased phosphorylation (inactivation) of pyruvate dehydrogenase. Genetic activation of glycolysis, with a *'glyco-high'* mutant construct, was sufficient to promote myofibroblast differentiation in WT fibroblasts. Conversely, genetic inhibition of glycolytic flux ablated the enhanced differentiation observed in *Mcu*$^{-/-}$ fibroblasts. Our results suggest that alterations in $_mCa^{2+}$ uptake and bioenergetic pathways are

necessary for myofibroblast differentiation. Thus, energetic signaling represents a novel therapeutic target to impede HF progression and other progressive fibrotic diseases.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA03

The structure of the mitochondrial permeability transition pore and design of novel inhibitors

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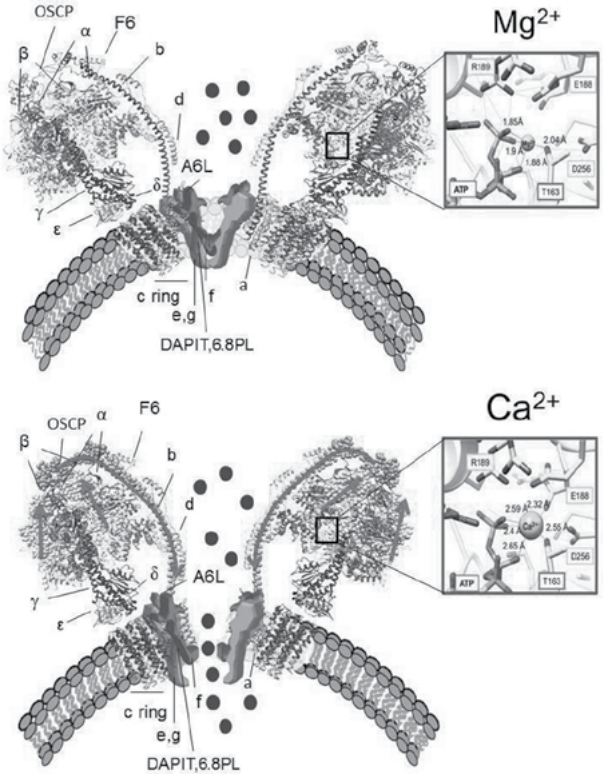
F- ATP synthases convert the energy of the transmembrane H^+ gradient into ATP with remarkable efficiency [1]. We have recently discovered that in the presence of Ca^{2+} F- ATP synthases can also form channels with the properties expected of the mitochondrial "permeability transition pore" (PTP) in mammals [2], yeast [3] and drosophila [4]. Ca^{2+}-dependent PTP opening causes a large increase of permeability of the inner mitochondrial membrane, which has long been known to dissipate ion gradients and to cause detrimental effects on mitochondrial and cell function [5]. Our results show that channel activity can be seen in reconstituted systems with highly purified F$_{_}$ ATP synthases, indicating that channel formation must occur within the enzyme complex. I will present our recent results on the mechanism of channel formation, as studied by site directed mutagenesis of key regulatory residues of F$_{_}$ ATP synthases, and on the development of new inhibitors.



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SA04

Mitochondrial trafficking in the brain

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Regulated trafficking of mitochondria in neurons is essential for providing ATP at the correct spatial location to power neural function and computation, and for providing calcium buffering at sites of calcium entry or release. Indeed the regulation of mitochondrial distribution, morphology and function are proposed to play an important role in neuronal development and survival but the regulatory mechanisms remain unclear. The proteins Miro1 and Miro2 contain a transmembrane domain locating them to the outer mitochondrial membrane, along with two GTPase domains and two calcium-sensing EF-hand domains that face into the cytosol, and play a key role in regulating mitochondrial transport. Miro proteins mediate mitochondrial trafficking and positioning in neurons by linking mitochondria to kinesin and dynein motor proteins for their transport in axons and dendrites. Miro proteins are also targets for the Parkinson's Disease associated PINK1/Parkin mitophagy pathway and are therefore implicated in altered mitochondrial dynamics during mitophagy. Here I will present our recent results on the role played by Miro proteins in controlling mitochondrial dynamics and reciprocally how this can influence neuronal development, synaptic function and pathology

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA05

Mitochondrial fusion dynamics in the skeletal muscle as a target of fibrosis

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Mitochondria are key players in the patho-physiology of skeletal and cardiac muscle. The quality control of mitochondria relays on fusion and fission processes. We demonstrated that the fusion of mitochondria is frequent in the skeletal muscle and determines excitation-contraction coupling (EEC), while in the heart, EEC-derived oscillatory calcium transients prompt mitochondria to undergo fusion. We also showed that chronic alcohol consumption is detrimental to mitochondrial fusion dynamics, both in heart and muscle. One of the hallmarks of alcoholic myopathy is fibrosis, an abnormal deposition of extracellular matrix, that leads to muscle dysfunction.
Currently, we are studying mitochondrial fusion dynamics in Duchene Muscular Dystrophy (DMD), a fibrotic paradigm. Particularly, we study the adult muscle

of WT and *mdx* mice ((6-9 months old), in a CTGF +/+ or +/- background. CTGF is one of the signals leading to accumulation of extracellular matrix. Skeletal muscle from mdx CTGF+/- mice shows inhibition of fibrosis and improved muscle function. We studied mitochondrial organization and fusion frequency in FDB (Flexor Digitorum Brevis) muscle isolated fibers, previously electroporated with mtDsRed and mtPA-GFP (photoswitchable form of GFP) expressing DNA plasmids, by means of in vivo confocal microscopy. Our data demonstrate that mdx-derived fibers, displayed disorganized topology and significant inhibition of mitochondrial fusion frequency, compared to WT fibers. Strikingly, mdx CTGF+/- restored mitochondrial organization pattern and fusion frequency to WT-like levels. In addition, mitochondrial fusion protein Mfn2 was significantly inhibited in mdx mice, however, CTGF+/- mdx prevented Mfn2 depletion. Furthermore, the ultrastructure of FDB muscles, tested by Transmission Electron Microscopy (TEM), showed highly disorganized topology and altered distribution of mitochondrial size, in the adult muscle of mdx mice, that was significantly restored in the CTGF +/- mdx genotype.
We then rescued Mfn2 protein in skeletal muscle of mdx mice, by acute exogenous electroporation. Expression of Mfn2 in FDB of mdx animals lead to recovery of mitochondrial fusion frequency to WT levels. Mitochondrial topology, number and size tested by TEM, was also restored. Finally, we tested the skeletal muscle strength development in tibialis anterior, finding partial restoration in mdx + Mfn2 muscles, in comparison to mdx skeletal muscle, without changes in the number of regenerative fibers or fibrosis.
Thus, our data suggests that fibrosis targets mitochondrial fusion dynamics and topology in a model of DMD, by inhibition of Mfn2. Genetic inhibition of fibrosis or recue of Mfn2 restores mitochondrial fusion and skeletal muscle function.

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SA06

Role of mitochondrial fusion proteins on liver metabolism.

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Mitochondrial fusion and fission are key processes regulating mitochondrial morphology. Mitochondrial fusion is catalyzed by Mfn1, Mfn2 and OPA1 in mammalian cells. Mfn1 and Mfn2 are transmembrane GTPases localized in the outer mitochondrial membrane and involved in tethering and fusion. Mfn2 protein seems to play a complex set of functional roles, and it also controls endoplasmic reticulum morphology and function.

Liver-specific ablation of Mfn2 in mice causes numerous metabolic abnormalities, characterized by glucose intolerance and enhanced hepatic gluconeogenesis. Mfn2 deficiency also impaired insulin signaling in liver. Furthermore, Mfn2 deficiency was associated with endoplasmic reticulum stress, enhanced hydrogen peroxide concentration, altered reactive oxygen species handling, and active JNK. In contrast, livers upon ablation of Mfn1 showed an enhanced mitochondrial respiration capacity and protection against insulin resistance induced by a high-fat diet. This pattern of changes suggests that Mfn1 and Mfn2 play distinct roles in hepatocytes or they induce an opposite pattern of adaptations upon loss-of-function.

I confirm that the above ethical and content criteria have been met and understand that The Society reserves the right to reject the abstract should it not conform to the above guidelines.

SA07

Mitochondria Fission in the dorsal vagal complex induces insulin resistance

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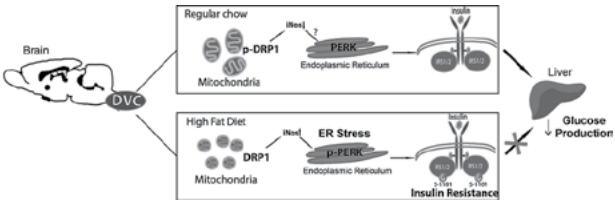
The central nervous system (CNS) integrates peripheral hormonal signals to regulate glucose homeostasis and feeding behavior. Obesity can cause the development of insulin resistance in the brain and completely disrupt the regulative functions of the CNS. In rodents, the Dorsal Vagal Complex (DVC) of the brain senses insulin to regulate glucose metabolism and 3 days high fat diet feeding (HFD) completely disrupts the insulin response. Mitochondria undergo morphological and metabolic changes to maintain energy homeostasis in eukaryotic cells. Whether specific changes of mitochondria dynamics directly alter cellular insulin signaling and whole-body glucose regulation remains unclear. We discovered that high-fat diet feeding in rodents decreases the phosphorylation levels of Dynamin-related Protein 1 (Drp1) in Ser-637, thus causing its activation and the consequent increase of mitochondrial fission in the DVC. This increase in mitochondria fission, in turn, increases iNOS levels, ER stress (seen as increase in p-PERK) and phosphorylation of IRS1 Ser-1101 that causes insulin resistance. Chemical inhibition of Drp1 by injecting Drp1 inhibitor MDIVI-1 in the DVC, inhibits mitochondrial fission, ER stress and phosphorylation of IRS1 Ser-1101 thus restoring DVC-insulin ability to lower hepatic glucose production *in* vivo. These results were also confirmed by expressing the dominant negative form of Drp1 in the DVC. Conversely, molecular activation of Drp1 in the DVC of healthy rodents is sufficient to induce DVC mitochondria fission, ER stress as well as insulin resistance. Taken together, these data illustrate that Drp1-dependent mitochondrial fission in the DVC is sufficient and necessary to induce insulin resistance and dysregulate hepatic

glucose production, and suggest that targeting the Drp1-mitochondrial-dependent pathways in the brain may carry therapeutic potential to reverse insulin resistance.



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SA08

Mitochondrial quality control in vivo

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Mitophagy, the autophagy of mitochondria, is thought to be an essential quality control (QC) mechanism of pathophysiological relevance in mammals. However, if and how mitophagy proceeds within specific cellular subtypes *in vivo* has remained unclear, largely due to a lack of tractable tools and models. To address this, we developed "*mito*-QC", a transgenic mouse with a pH-sensitive fluorescent mitochondrial signal. This allows the assessment of mitophagy and mitochondrial architecture *in vivo*. *mito*-QC revealed that mitophagy is a significant process in most tissues, even under basal conditions. However, within tissues it can be spatially restricted to distinct cell types, particularly those with high metabolic demands such as photoreceptor cells in the retina or proximal tubule cells in the kidney. Surprisingly, the mechanisms behind these instances of basal mitophagy are distinct from the classical starvation-induced autophagy pathway and do not require activation of PINK1, which previously was thought to be the key mitophagy-stimulating event.

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SA09

Autophagy-mitochondria axis in health in disease

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Autophagy has multiple cellular functions and is important for the maintenance of proteome, genome and organellar homeostasis. Perturbation of autophagy has been associated with multiple pathologies however it remains unclear how different cellular roles of autophagy contribute to the cellular dysfunction in conditions of autophagy impairment. Our data suggest specific mechanisms by which autophagy deficit and an impairment of mitochondrial quality control by autophagy (mitophagy) may lead to cell death. We use a lysosomal storage disease model characterised by autophagy dysfunction as a proof of principle for the relevance of these mechanisms to human neurodegenerative diseases.

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SA10

Mitochondrial remodeling: Rearranging, recycling, and reprogramming

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Mitochondria, in keeping with their endosymbiont origins, are highly dynamic organelles, undergoing fusion and fission. Moreover, they are subject to cellular processes such as elimination via mitophagy or through ejection as exosomal particles. Mitochondrial mass is maintained at tissue-specific levels through biogenesis, a process that requires coordinated nuclear and mitochondrial gene expression and protein synthesis. Nuclear-encoded proteins are co-translationally imported from cytosol to mitochondria, a process depending on the complex protein import machinery that spans both mitochondrial outer and inner membranes. In response to stress such as ischemia/reperfusion injury, mitophagy is activated. In the heart, this process is Parkin-dependent but also involves optineurin, NDP52, and processing of Opa1. Homeostatic mechanisms are then initiated to restore mitochondrial mass to its original level, involving mtDNA replication and synthesis of both nuclear- and mitochondrial-encoded proteins. We are studying this process in the human heart and have found that during cardiac surgery involving cold cardioplegia there is evidence for both mitophagy and biogenesis. Moreover, we have preliminary evidence that this is regulated in part by miRNAs that suppress mito-biogenesis under normal conditions; depletion of these miRNAs from cytosolic polyribosomes occurs when mito-targeted protein translation is increased.

Using Langendorff-perfused mouse hearts subjected to ischemia and reperfusion, we have identified an increase of mitochondria-targeted nuclear-encoded mRNAs associated with polysomes despite no change in total mRNA levels for these genes. Proteomic analysis of polysomes isolated by detergent extraction and sucrose gradient centrifugation revealed ribosomal proteins as well as numerous outer and inner membrane components of the mitochondrial protein import machinery, providing new evidence supporting co-translational protein import. Work is ongoing to identify the miRNAs that participate in this process in the mouse Langendorff model and to identify the newly-synthesized proteins.

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SA11

The Mitochondria Reticulum of Muscle Cells

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Recently both 3D high resolution and functional studies in muscle cells have revealed a tightly coupled mitochondria reticulum (MR) to rapidly distribute potential energy, in the form of the mitochondrial membrane potential (MMP), throughout skeletal muscle and heart cells. Herein the structural aspects of the MR are described using 3D Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and presented in muscle cells. A large portion of the MR conductivity is dependent on direct mitochondrial matrix continuity while in some regions of the muscle the connectivity is proposed to occur via poorly characterized electron dense regions (EDR) between adjacent mitochondria. Using a photo-activated mitochondrial uncoupler to regionally perturb the MMP, we have demonstrated that large regions of the MR are electrically coupled via a shared matrix as well as EDR structures between the mitochondria. In murine skeletal muscle cells a large fraction of the mitochondrial volume is located in regions close to capillary indentations in the cell structure. These embedded capillaries are surrounded by large pools of mitochondria near the plasma membrane that have narrow tubes which run along the I-Bands (I-Band Mitochondria Segments (IBMS)) deep into the muscle cell. It has been proposed that these IBMS serve to distribute the MMP from the large sub-sarcolemma mitochondrial pool to the more central ATP-consuming myofibril region of the muscle cell. Consistent with this notion was the observation that there is a 3-fold enhancement of MMP generating oxidative phosphorylation complex in comparison to MMP utilizing ATP synthesis enzymes in the periphery of the muscle cell when

compared to central regions near the muscle ATPase activity. In cardiac cells, no IBMS exist and the coupling is exclusively through large mitochondria structures and numerous EDR connections. These data are consistent with a mitochondria reticulum in muscle cells that couples large numbers of mitochondria together providing a rapid and uniform potential energy source throughout the cell to support ATP production. (NHLBI Division of Intramural Research).

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SA12

Exercise and skeletal muscle mitochondria

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Skeletal muscle mitochondria are highly adaptable and respond to exercise training by multiple mechanisms. In humans, exercise modulates the different electron transport chain complexes distinctively. Among the molecular adaptations, exercise favors the shift of free complexes into functional supercomplexes assemblies. Free and superassembled complexes co-exist and are recruited in response to energy demand. The content of supercomplexes is positively related to muscle respiration and exercise efficiency. Other long-term adaptations to exercise include the improvement of mitochondrial quality through mitochondrial turnover and adaptations of mitochondrial dynamics.

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SA13

The Effect of Maternal Diet & Obesity in Pregnancy on Mitochondrial Function in the Offspring

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Maternal obesity and/or a poor nutritional diet during pregnancy is proposed to alter the programming of metabolic systems in the offspring, increasing the risk for developing metabolic diseases; however, the cellular mechanisms are still relatively unknown. Here, we used a nonhuman primate model to examine the impact of a maternal Western-style diet (WSD) alone, or in combination with obesity (Ob/WSD), on offspring skeletal muscle metabolism studied in early third trimester fetus and in lean juvenile (peripubertal) offspring at 3 years of age. We also tested the benefits of a healthy diet intervention initiated either at the start of pregnancy in obese dams or

after weaning in juvenile offspring on offspring metabolic outcomes. Group-housed adult Japanese macaques were fed a control (CTR) or WSD for at least two years prior to the start of the study. A subset of obese dams that had been chronically fed WSD were switched to the healthy CTR diet at the start of pregnancy (Ob/DR). Pregnancies were terminated at gestational day 130/165 and fetal muscles collected (Ln/CTR, n=24; Ln/WSD, n=17; Ob/WSD, n=26; Ob/DR, n=6). A second cohort of offspring from Ln and Ob dams were taken to term, weaned at 7 months, and fed either the same maternal diet or switched to a the opposite diet (n=6-8). Substrate utilization and respiratory capacity were measured in permeabilized muscle fiber bundles (PMFBs) from fetal and juvenile muscle by high resolution respirometry (O2K, Oroboros). Data were analyzed by 1-way ANOVA in fetal muscle and 2-way ANOVA in juvenile muscle (maternal diet x postweaning diet). We find that fetal muscle adapts to maternal Ob/WSD by down-regulating glucose utilization and enhancing the capacity for fatty acid oxidation. Specifically, there was an increase in very-long-chain acyl-CoA dehydrogenase (VLCAD; 50%), carnitine palmitoyltransferase 1β abundance (CPT1β; 2-fold) and pyruvate dehydrogenase kinase 4 (*PDK4;* 2-fold) expression in Ob/WSD compared to Ln/CTR ($P \leq 0.01$). Mitochondrial complex (C)-I and C-IV activities were also increased 2- and 3-fold in fetal muscle of Ob/WSD vs. Ln/CTR despite a decrease in markers of mitochondrial content. Muscle triglyceride accumulation was reduced by 50% in Ob/WSD ($P<0.0001$). Although, fetal muscle from Ln/WSD showed only intermediary changes in metabolic markers, maternal WSD alone was sufficient to increase oxidative damage (TBARs) and expression of markers of cellular stress (Gadd45, UCP2/3, SIRT1). In fetal PMFB, total oxidative capacity (20% lower), uncoupled ETS capacity (28%) and ETS coupling efficiency (15%) were significantly lower in Ob/WSD compared to Ln/CTR using non-lipid substrates ($P<0.0001$); addition of palmitoylcarnitine partially restored the decrease in oxidative capacity corroborating molecular data for increased lipid utilization. Switching obese dams to healthy diet during pregnancy further reduced both lipid and non-lipid oxidative metabolism and mitochondrial efficiency in PMFBs from fetal Ob/DR compared to Ob/WSD. Despite increased lipid utilization in fetal muscle, in PMFB of juvenile offspring (36 months) from Ln/WSD, there was an ~80% decrease in fatty acid oxidation (interaction; $P<0.0001$) and CI-linked oxidative capacity (interaction; $P=0.01$) independent of post-weaning diet. A similar decrease in fatty acid oxidation (but not CI- or total oxidative capacity) was also found in PMFB from offspring of Ln/CTR dams fed a post-weaning WSD. There was no effect of maternal WSD or post-weaning WSD on non-lipid oxidative metabolism or in citrate synthase activity in juvenile muscle. Maternal obesity/WSD leads to an early up-regulation in lipid oxidation and increased cellular stress in fetal muscle. These fetal adaptations likely contribute to the decrease in lipid oxidative metabolism in juvenile muscle and may increase the susceptibility to metabolic diseases in this population. Lastly, in these cohorts, we find that a healthy diet intervention alone, initiated in an obese pregnancy or at weaning, was insufficient to fully reverse the effects of maternal obesity or WSD, respectively on offspring mitochondrial metabolism.

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C01

Ca^{2+}-induced mitochondrial ROS regulate the early cell cycle in Xenopus embryos

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Reactive oxygen species (ROS) act as second messengers in both homeostastic and stress response signaling pathways. The roles for ROS during early vertebrate development, however, have remained largely unknown. Using *Xenopus* frog embryos and a genetically encoded ROS indicator HyPer, we found that oocyte fertilization induces an increased production of ROS that is sustained during early development, with oscillations associated with each cell division. We show that fertilization induced ROS production is dependent upon Ca^{2+} signaling and, consistently, that Ca^{2+} ionophores are sufficient to induce ROS production in unfertilized oocytes. Using chemical inhibitors, we identify mitochondria as the major source of fertilization induced ROS production. Inhibiting mitochondrial ROS production causes a misregulation of Cdc25C phosphatase, resulting in cell cycle arrest during the early cleavage stages. Thus, our study reveals an important role of ROS interlinking the Ca^{2+} wave at fertilization and the cell cycle oscillator in *Xenopus* embryos.

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C02

GBA1 deficiency sensitises neurons to glutamate toxicity and causes calcium dyshomeostasis

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Glucocerebrosidase (GBA1) is a lysosomal enzyme whose homozygous mutations cause Gaucher's disease (GD), a lysosomal storage disorder. Severity and symptoms are variable but include a severe neurodegenerative 'neuropathic' form. Heterozygous mutations in GBA1 are the major known genetic risk factor for Parkinson's disease (PD). The deficiency of GBA1 causes impaired autophagy but was also associated with reduced mitochondrial membrane potential, mitochondrial fragmentation and respiratory chain defects (1). Since mitochondrial dysfunction

can impact on free radical production and calcium homeostasis, we used fluorescence imaging microscopy and biochemical approaches to investigate these variables in neurons cultured from a GBA1 knockout (KO) mouse stimulated with a non-toxic glutamate concentration (10μM).

We found that basal rates of reactive oxygen species (ROS) (measured by imaging the dye dihydroethidium) are increased in GBA1 KO neurons compared to wild-type (WT) and heterozygous (HET) neurons in basal conditions, while all the genotypes responded similarly to glutamate, showing an increase in the rates of ROS generation. The increased free radical production did not correspond to a change in the expression of the antioxidant proteins superoxide dismutases 1 and 2.

The calcium responses to glutamate showed an immediate 'early' response to 10μM glutamate stimulation which was significantly higher in HET and KO neurons compared to WT, measured by Fura2 imaging. Moreover, a higher proportion of HET and KO neurons showed a late increase in cytosolic calcium termed 'delayed calcium deregulation (DCD)' that is usually seen only at higher glutamate concentration in WT neurons (>100μM) and leads to cell death.

This was associated with dysregulation of mitochondrial calcium handling: measurements of mitochondrial calcium uptake (using mitochondrial targeted aequorin) showed a decrease in response in HET and KO compared to WT cells. This was also associated with a reduced expression of the mitochondrial calcium uniporter (MCU), which is the channel responsible for the mitochondrial calcium uptake.

Expression levels of glutamate receptors (glutamate ionotropic receptor kainate type subunit 4 and glutamate ionotropic receptor NMDA type subunit 2B) were not changed at the transcript level (as probed by pQCR) showing that the increased response to glutamate in GBA1 KO cells was not due to their altered expression.

These data together suggest calcium handling is dysregulated in GBA1 KO neurons, with an increased sensitivity to otherwise innocuous glutamate concentrations, a mechanism which may contribute to neurodegeneration in GD. Strikingly, HET neurons showed a behavior that was more similar to KO rather than to WT, consistent with a role of these mechanisms in the pathogenesis of PD.

Osellame LD et al (2013). *Cell Metab* **17**, 941-953.

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C03

Amyloid-β oligomers or hyperglycaemia each reduce mitochondrial motility in mature hippocampal cells

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Amyloid-β plaques are indicative of Alzheimer's disease. A precursor of these plaques: oligomers consisting of a few amyloid-β peptides, may be sufficient to impair brain function, partially by impairing the trafficking of mitochondria[1]. Hyperglycaemia may also decrease axonal mitochondrial motility[2] and contribute to the risk of developing neurodegenerative disease, thus we sought to examine how both high glucose and amyloid-β oligomers influence mitochondrial motility.

Complex, overlapping mitochondrial morphologies make analysis of mitochondrial dynamics challenging. We have developed image analysis techniques that enable the discrimination of individual mitochondria within optically-crowded environments[3] and can track mitochondria to sub-pixel resolution[4]. Primary hippocampal cell cultures from p0-2 rat pups were grown in either normo- or hyper-glycaemic media (3 or 25 mM glucose) for 3 weeks then loaded with tetramethylrhodamine ethyl-ester plus Mitotracker-Green-FM (100 nM each) prior to epifluorescence imaging for 5 min. The cells were then incubated with either 5 μM amyloid-$\beta_{1\text{-}42}$ oligomers or the reversed peptide (42-1) or vehicle (DMSO, 0.1%) for 1 hr prior to a second period of imaging. In these mature cells amyloid-β oligomers, but not reversed peptide or vehicle, caused a decrease in mitochondrial motility of cells grown in either low or high glucose. Additionally, for cells grown for 3 weeks prior to imaging, mitochondrial motility was lower in high-glucose media (4.76±1.53%, mean±S.D., total mitochondrial area moved min^{-1}, n=9, compared to 8.04±1.54% in normo-glycaemia, n=11 coverslips of cells from ≥4 independent preparations each; p=0.012, one-way ANOVA with Tukey's post-hoc test). There was no difference in mitochondrial motility due to media glucose concentration in youngers cells, however (1 week: 8.03±3.13% total mitochondrial area moved min^{-1} in normo-glycaemia, n=14, c.f. 6.45±1.89% min^{-1} in hyper-glycaemia, n=15; 2 weeks: 6.37±1.56% total mitochondrial area moved min^{-1} in normo-glycaemia, n=15, c.f. 6.78±2.4% min^{-1} in hyper-glycaemia, n=10). A wide range of mitochondrial morphologies were observed in all cell preparations and glucose concentrations, however there was a shift towards more small, punctuate mitochondria in hyper-glycaemia, with no difference in morphology observed due to amyloid-β oligomers.

In summary, hyperglycaemia caused a decrease in mitochondrial motility and size in mature (but not immature) cultured hippocampal cells. Acute exposure of mature hippocampal cells to amyloid-β oligomers caused a decrease in mitochondrial motility, both in normo-glycaemic and hyper-glycaemic conditions. Thus

both amyloid-β oligomers and hyper-glycaemia may contributing to neuronal vulnerability by altering the involvement of mitochondria in calcium buffering or interactions with structures such as the endoplasmic reticulum.

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4: Chalmers, S *et al*. (2012) *Arterioscler Thromb Vasc Biol 32*;3000-11

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C04

Assessing mitochondrial structure and function in C. elegans muscle

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Caenorhabditis elegans is a small laboratory animal commonly used for genomics studies. It has also been used for landmark studies of development, signal transduction, gene silencing, and ageing as well as clinically relevant studies of mitochondrial disease. We have been using this worm to study genes and signals that regulate muscle homeostasis. In our studies of "muscle genes", kinases, and phosphatases that appear to be required for muscle homeostasis, we discovered that mitochondrial structure, as assessed by GFP labelled mitochondria, was much more frequently disrupted than sarcomere structure; **NB.** these observations represents knockdown of roughly 10% of the total genome. Therefore, we have been using a number of techniques to assess mitochondrial structure and function in *C. elegans* muscle. As with many systems, MitoTracker dyes can be used to assess mitochondrial structure and function in all tissues, both *in vivo* and via fluorescence-activated cell sorting (FACS) based separation. Using FACS, we find that muscle mitochondria comprise roughly 20% of all mitochondria in *C. elegans*. We have used similar dyes such as JC-1 to show progressive loss of mitochondrial membrane potential *in vivo* in response to genetically locking open a degenerin cation channel, UNC-105, in muscle. In muscular dystrophy mutants we have employed the Seahorse instrument to demonstrate alterations in oxygen consumption. We have also combined these techniques and others, such as citrate synthase activity and maximal rates of ATP synthesis, to study processes of physiologic interest. For example, we find that mitochondrial structure is an early event in ageing muscle

and this is preceded by decreases in mitochondrial function. Lastly, we have begun to use these techniques to assess the impact of compounds that may improve mitochondrial structure and function. For example, ongoing studies suggest that GYY4137 improves mitochondrial structure with age and mitochondrial function in muscular dystrophy. By having applied a variety of techniques, we feel that we are now able to get a comprehensive view of mitochondrial structural and functional capacity in *C. elegans* and use this worm in basic, applied, and translational mitochondrial research.

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C05

Components of the innate immune system evoke mitochondrial membrane hyperpolarisation, increases in mitochondrial reactive oxygen species via Nox and induce cell death in the renal medulla.

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Background: The innate immune system, mitochondrial membrane potential ($\Delta\Psi m$), reactive oxygen species (ROS) and cell death are all thought to be involved in the pathogenesis of acute kidney injury (AKI) [1]. How these processes are linked however, remains unclear. AKI is sudden damage to the kidneys, which in the absence of specific therapy can lead to severe kidney damage and mortality [2]. Understanding the cellular mechanisms involved in AKI may help improve diagnostics and provide novel therapeutic targets for the treatment of AKI after ischemic injury.
Method: A rat 'live' kidney slice model [3] was used in combination with multiphoton microscopy to investigate the effect of components of the innate immune system on mitochondrial membrane potential, mitochondrial ROS production and cell death in the renal medulla. Kidney slices were loaded with the appropriate fluorescent dye; tetramethylrhodamine methyl ester (TMRM), mitoSOX and propidium iodide (PI), respectively prior to exposure to C5a, IL-33, TNF-α or IL-18 for 10 minutes. Real time changes in fluorescence was recorded throughout experiments and analysed off line. All data are mean ± S.E.M, n≥6 animals. Significance was determined using Students' t-test and all $p \leq 0.05$.
Results: C5a evoked a significant increase in TMRM signal in endothelial (E; 34.73% ± 4.56%,), pericyte (P; 40.87% ± 8.34%) and tubule cells (T; 34.40% ± 7.12%). As did IL-33 (E: 26.25% ± 2.68%, P: 29.27% ± 8.82, T: 20.79% ± 2.61%), IL-18 (E: 31.61% ± 4.76%, P: 32.66% ± 2.95%, T: 23.94% ± 1.57%) and TNF-α (E: 44.72% ± 3.72%, P: 52.64% ± 4.29%, T: 54.75% ± 6.14%).

C5a also evoked a significant increase in MitoSOX signal in endothelial (E; 24.96% ± 5.87%), pericyte (P; 31.41% ± 7.70%,) and tubule (T; 50.14% ± 7.69%) cells. As did IL-33 (E: 44.59% ± 8.59%, P: 30.92% ± 9.07%, T: 26.25% ± 3.37%), IL-18 (E: 54.05% ± 3.46%, P: 56.56% ± 9.75%, T: 69.60% ± 5.17%) and TNF-α (E: 61.10% ± 9.72%, P: 51.51% ± 12.08%, T: 70.41% ± 9.25%). When tissue was exposed to innate immune components in combination with a Nox inhibitor apocynin, the innate immune component-induced increases in MitoSOX fluorescence was significantly attenuated in all cases.
C5a, IL-33, IL-18 and TNF-α all induced significant increases (22.74% ± 4.80%, 15.58% ± 3.81%, 11.79% ± 1.93%, 17.02% ± 4.78%) in dead cells labelled with PI across the kidney slice.
Conclusion: Innate immune system components C5a, IL-33, IL-18 and TNF-α all act at medullary endothelial, pericyte and tubule cells to cause mitochondrial hyperpolarisation and increases Nox-dependent mitochondrial ROS, that are linked to renal cell death. This data suggest alterations in mitochondria function are likely to be key in immune-mediated pathophysiology associated with AKI.

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C06

Krebs cycle intermediates drive abnormal metabolic signalling in response to hypoxia in the diabetic heart

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Hypoxia-inducible factor 1α (HIF1α) is activated following myocardial infarction, and is critical for cell survival in hypoxia. In cancer, changes in Krebs cycle intermediates have also been shown to modify HIF1α stabilisation. We have previously shown that type 2 diabetic hearts have decreased tolerance of hypoxia. We questioned whether abnormal metabolism could prevent HIF1α activation in diabetes, and whether Krebs cycle intermediates were mediating this maladaptation.

Methods: Type 2 diabetes was generated in male Wistar rats by 6 weeks of high fat feeding in combination with a low dose intraperitoneal injection of streptozotocin (25mg/kg) after 2 weeks. Rats were terminally anaesthetized using an intraperitoneal injection of sodium pentobarbital (150 mg/kg bodyweight, Euthatal). Insulin resistance was also modelled in cell culture, using HL1-cardiomyocytes cultured with glucose, lipids and insulin.
Results: Type 2 diabetic hearts have decreased HIF1α protein accumulation following myocardial ischemia, which correlated negatively with plasma fatty acid concentrations and positively with myocardial succinate concentrations. In insulin-resistant cardiomyocytes, HIF signalling and downstream metabolic adaptation was suppressed in response to hypoxia. Impaired HIF1α stabilisation was due to increased degradation of the protein in hypoxia, as inhibition of the proteasome or inhibition of the regulatory HIF hydroxylases was able to increase HIF1α. Increased HIF1α degradation in diabetes was due to abnormal metabolism. We have found that long chain fatty acids prevented HIF1α accumulation in a concentration-dependent manner, which could be reversed by blocking fatty acid uptake. Succinate promotes HIF stabilisation by inhibiting the HIF hydroxylases, however, we have found that fatty acids suppressed succinate accumulation during hypoxia. Supplementing succinate concentrations in cells overrides the fatty acid-mediated inhibition of HIF1α, in a concentration-dependent manner. Finally, pharmacologically inhibiting the HIF hydroxylases promoted HIF1α accumulation and improved cardiac function following ischemia-reperfusion in diabetic rats.
In conclusion, elevated fatty acids in type 2 diabetes prevent HIF1α accumulation by decreasing succinate concentrations in hypoxia.

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C07

Low levels of the A3243G mitochondrial DNA mutation in human induced pluripotent stem cell-cardiomyocytes do not cause functional or metabolic disturbances but increase with further passaging

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The heteroplasmic mitochondrial DNA (mtDNA) mutation A3243G can cause the mitochondrial condition Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). Mitochondrial replacement therapy can prevent transmission of mtDNA mutations to offspring but to maintain nuclear integrity, a certain amount of cytoplasm and mutated mtDNA is carried over (< 3%). It is unknown whether this will increase with age and this is particularly relevant in the

heart, where mutations accumulate over time. We applied small molecule modulation of the Wnt/β-catenin signalling pathway to generate pure populations of cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) from a patient with 20% heteroplasmy for the A3243G mtDNA mutation. No changes in the basal beating rate or time to peak and time to 50% relaxation were found. No differences in the response to β-adrenergic stimulation by isoprenaline or muscarinic inhibition by carbachol. A3243G hiPSC-CMs showed reduced excitability (18.85 ± 3.045 ms for control and 38.08 ± 6 .126 ms for A3243G, Mean ± SEM, n=17, p= 0.0084, Unpaired t-test) but there were no changes in other calcium handling properties. Mitochondrial DNA copy number and both mitochondrial respiration and basal glycolysis were unaffected. We have seen a gradual increase in A3243G hiPSCs and derived CMs heteroplasmy with passaging (26.4 to 38.7 % over 6 passages). We conclude that A3243G heteroplasmy <40% is not sufficient to affect the generation of hiPSC-CMs and their beating, calcium handling and metabolic properties. Having observed an increase in heteroplasmy with passaging, these results provide useful insights into changes that might happen with age in patients with the A3243G mutation and in children resulting from mitochondrial replacement therapy.

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C08

Physiology and pathology of alpha-synuclein

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Alpha-synuclein aggregation and mitochondrial dysfunction are central to the pathogenesis of Parkinson's disease (PD). Despite a wealth of publications about misfolded α-synuclein in Parkinson's disease, very little has been reported about this protein's physiological function in health and the mechanism of its toxic gain of function in disease. This study establishes an important function for monomeric α-synuclein in the regulation of ATP synthase activity as application of extracellular monomeric α-synuclein shows that monomers enter the cell and localise to mitochondria, interact with ATP synthase and aids its efficiency (1). In Parkinson's disease, the monomer aggregates to form toxic oligomers, and we show that these aggregates maintain an interaction with the ATP synthase, a proposed key component of the mitochondrial permeability transition pore (mPTP). Oligomers, but not monomers, directly induce mPTP, and lower the threshold for calcium induced and ROS induced mPTP opening. Oligomeric α-synuclein is uniquely redox active and its ability to interact with ATP synthase and surrounding mitochondrial membranes, selectively oxidise its target. This targeted oxidation induces its conversion to form the mPTP leading to cell death which can be prevented by inhibition of the

oligomer-induced oxidation events. This study highlights a physiological effect of monomers and the mechanism by which conformational changes (of α-synuclein) exert a toxic gain of function leading to neurodegeneration.

Ludtmann M.H.R., Angelova, P.R., Ninkina, N., Gandhi S., Buchman V.L, Abramov A.Y. (2016) Monomeric alpha-synuclein exerts a physiological role in brain ATP synthase. Journal of Neuroscience, 36 (41) 10510-10521

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C09

Preventing Glyoxalase-1 (Glo-I) downregulation attenuates mitochondria dysfunction in hearts of diabetic rats

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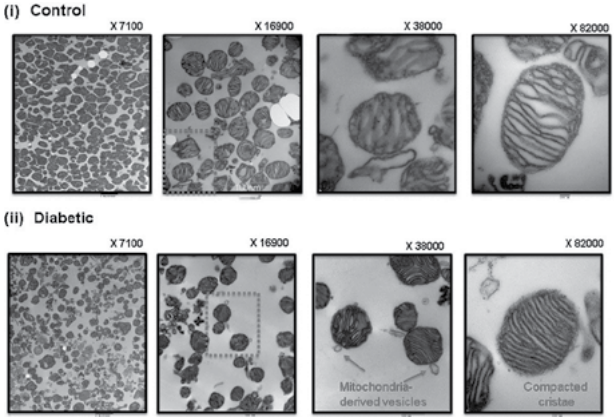
Diabetic heart failure (dHF) is an established cause of morbidity and mortality in individuals with diabetes mellitus (DM). Supra-physiologic production/flux of the alpha-oxoaldehyde methylglyoxal (MG) has emerged as likely candidate since it can promote mitochondrial dysfunction, a known cause of dHF. In this study which was approved by the Animal Care and Use Committee, University of Nebraska Medical Center, a custom-designed adeno-associated virus in conjunction with echocardiography, electron, atomic force and electro-paramagnetic resonance spectroscopies, live cell imaging, Western blot analyses and high performance liquid chromatography was used to determine if preventing supra-physiologic MG in DM rat hearts is mitochondria and cardio-protective. After eight weeks of streptozotocin-induced DM, E:A ratio was increased and % fractional shortening and ejection fraction were reduced in 3-4% isoflurane-anesthetized rats, indicative of dHF. Rats were then sacrificed by injecting with Inactin (Sodium thiopental, 120 mg/kg, i.p) and hearts were harvested. In ultrathin ventricular sections from DM hearts, SSM were no longer thread-like but disjointed from each other and migrated away from the plasma membrane (~5 nm). Purified SSM from DM hearts were also smaller (850 ± 20 nm, compared to 1090 ± 25 nm for control) with more compact, lamelliform cristae (21 ± 4 nm compared to 28 ± 3 nm for control), and generated ~2.5X higher basal reactive oxygen species (ROS)/μg mitochondria. Isolated SSM and SSM in ventricular tissues also contained single and double membraned mitochondrial-derived vesicles, suggesting extrusion of oxidized cargoes. Isolated SSM also contained ~50% less connexin 43 and superoxide dismutase-1 (SOD-1), and ~ 2X more dynamin-1-like protein (Drp-1) and mitofusin II (mfn-2). NDUF2, 3 and 10 contained ~ 2-3X more MG adduct on them compared to controls. Preventing Glo-I downregulation in DM rats by administering an AAV2/9 containing Glo-I

driven by the endothelin-1 promoter one week after the onset of DM, attenuated impairments in E:A ratio, % fractional shortening and ejection fraction later in the disease (7-8 weeks). It also attenuated changes in structure and function of SSM induced by DM. These data show for the first time that supra-physiologic MG flux is an underlying cause of SSM dysregulation in hearts of DM rats. They also show that attenuating supra-physiologic MG flux by preventing Glo-I downregulation is sufficient to blunt SSM dysregulation and HF in DM.



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C10

Evaluation of a novel mitochondria-targeted peptide based H_2S donor compound (RTP-10) in hyperglycaemia-induced microvascular endothelial cell dysfunction

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An overproduction of mitochondrial reactive oxygen species (ROS) in endothelial cells, is a major contributor to vascular endothelial dysfunction (VED) and angiopathy in diabetes [1]. Hyperglycaemia (HG) induces metabolic changes in mitochondria, notably superoxide production, membrane hyperpolarisation and loss of ATP synthesis [2]. Supplementation of cells and diabetic animals with sources of hydrogen sulfide (H_2S) has been shown to protects mitochondria from the detrimental effects of oxidative stress (e.g. in hypoxia/ischaemia-reperfusion injury,

diabetic angiopathy, stroke etc.) as well as reverse vascular damage in animal models of diabetes in *vivo* [3]. Moreover H_2S has been shown to prevent HG-induced metabolic changes in vascular endothelial cells.

Given the predominantly mitochondrial effects of H_2S against diabetic vascular damage, we previously described two mitochondria-targeted H_2S (mtH_2SD) donor organic compounds based around a triphenylphosphonium (TPP^+) targeting scaffold, AP39 [4] and AP123 [5]. These compounds have shown significant efficacy in animal models of mitochondrial dysfunction at very low doses (7-300 μg / kg). In this current study, we have used an alternative approach to target H_2S to mitochondria using a novel H_2S donor derivative of D-Arg-L-Tyr-L-Lys-L-Phe-NH_2 (RTP-10). This approach may be advantageous over TPP^+-based scaffolds as mitochondrial accumulation is not dependent on mitochondrial $\Delta\Psi m$. We therefore exposed murine B.End3 brain microvascular endothelial cells to hyperglycaemia (HG) and after 7 days added RTP-10 and measured the reversal of HG-induced metabolic changes, specifically mitochondrial $\Delta\Psi m$ (JC-1), mitochondrial superoxide (mitosox), ATP synthesis (by luminescence). RTP-10 caused a concentration-dependent (0.1-30 μM) increase in mitochondrial H_2S levels and reversed HG-induced mitochondrial hyperpolarisation and oxidant production, and restored ATP synthesis. Our study further suggests that targeting H_2S to mitochondria may be a useful therapeutic strategy for preventing/inhibiting HG-induced VED and angiopathy.

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Brian Ridge Scholarship, Medical Research Council, UK (MR/M022706/1).

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C11

Type 2 diabetic mitochondria overcome fatty acyl CoA inhibition, but have reduced respiration

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Introduction: The type 2 diabetic mellitus (T2DM) heart is energetically dysfunctional as seen by a reduced phosphocreatine/adenosine triphosphate (ATP) ratio in humans. The vast majority (95%) of cardiac ATP is generated in the mitochondria through oxidative phosphorylation, making them the most likely candidate for energetic dysfunction. One protein stands out as the key regulatory point in oxidative phosphorylation, with over 70% of control; the adenine nucleotide translocase (ANT). This protein is potently inhibited by fatty acyl CoAs which may link the high fat phenotype and defective energetics of the T2DM heart. We tested whether dysfunctional energetics in the T2DM heart could be traced to altered kinetics around the ANT.
Methods: Type 2 diabetes was induced in Wistar rats by feeding of a high fat diet for 7 weeks. Two weeks into this, rats were given a low dose injection of streptozotocin. At 7 weeks, rats were sacrificed under terminal anaesthesia (overdose of isoflurane inhalation). Cardiac subsarcolemmal and interfibrillar mitochondria were isolated from diabetic and control rat hearts. Mitochondrial respiration was measured using a Clark type oxygen electrode.
Results: The presence of palmitoyl CoA (P-CoA) competitively inhibited respiration in control mitochondria, shown by a 2.5 fold (136.1±15.7 vs 57.8±8.1) increase in Km in the presence of P-CoA. This was eliminated by uncoupling the mitochondria, demonstrating that inhibition was centred on the phosphorylation apparatus. Diabetic mitochondria were far more resilient to fatty acyl CoA inhibition with no significant increase in Km (82.4±7.3 vs 65.1±6.9). Diabetic mitochondria had a 20% reduction in ADP stimulated respiration compared to controls (110.5±7.8 vs 78.2±5.9) when respired in the absence of fatty acids (on glutamate, pyruvate and malate). There was a very strong correlation ($P<0.005$) between sensitivity to fatty acyl CoA, and ADP stimulated respiration, indicating that the two phenomena may have the same cause. Despite these phenomena being intrinsically linked by the ANT, there was no change to either ANT1 or 2 expression in diabetic mitochondria (as measured by Western blotting (n=7)).
Conclusion: Type 2 diabetic mitochondria showed differential kinetics around the ANT for both P-CoA regulation, and adenine nucleotide transport (as measured by respiratory rates) despite no change to either ANT 1 or 2 expression. Together these data indicate that kinetics around the ANT, the key protein in respiratory control are altered in type 2 diabetes. This provides a potential cause for the dysfunctional energetics observed in the type 2 diabetic heart.

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PC01

Exosomal Transfer of MicroRNA in Vascular Smooth Muscle Cell Proliferation is Mitochondrial-Dependent

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Exosomes are widely recognised as important autocrine/ paracrine mediators of cell function. Vascular smooth muscle (VSM) cells undergo phenotypic transformation from a contractile to a synthetic hyperproliferative phenotype in atherosclerosis and re-stenosis associated with percutaneous coronary interventions. We have previously shown mitochondrial bioenergetics are upregulated in synthetic hyperproliferative VSM cells. In this work we aimed to study potential correlation between the synthetic hyperproliferative VSM cell and exosomal-dependent signalling.

All experiments were undertaken in VSM cells isolated from aorta harvested from 12 week old male Sprague Dawley rats. Exosomes were isolated from VSM cells using a sucrose gradient-ultracentrifuge technique and confirmed by zestier and western blotting for CD markers. miRNA and RNA measured using standard qRT-PCR. Cell proliferation measured using 3H thymidine incorporation.

Total exosomal release was increased in synthetic VSM cells vs. wild type (WT) cells. Likewise, total protein and RNA within exosomes in synthetic VSM was significantly greater when compared with WT VSM cells (Table 1.). Addition of exosomes isolated from WT or synthetic hyperproliferative cells to VSM cell cultures resulted in 4.7 ± 2.3% and 23.4 ± 4.6% respectively, $p<0.05$. qRT-PCR of exosomal contents (synthetic VSM cells vs. WT VSM cells) highlighted a significant reduction in pro-apoptotic genes (Bnip3, SOD1, SOD2), a reduction in significant tumour suppressor genes (Pmaip1, p53) and significant reduction in cell cycle regulator Cdkn2a. Likewise, RNA for PI3K, 4EBP1 and mTOR were all significantly greater in exosomes isolated from synthetic VSM cell vs. WT VSM cells. miRNA sequencing highlighted significant differences in exosome contents. Notably a 10-fold increase in pro-mitogenic miR21 and 3.5-fold loss of anti-mitogenic miR145, synthetic VSM cell vs. WT VSM cells. The use of selective anti-miRs/ miR mimetics in cell proliferation assays confirmed that both miR145/ miR21 are regulators of VSM cell phenotype and proliferation. Inhibition of mitochondrial bioenergetics or mitochondrial dynamics (fission-fusion) with the DRP-1 inhibitor MDivi-1 normalised exosomal yield, exosomal contents, RNA and miRNA similar to that measured in WT cells.

Our results implicate exosomes and their RNA/ miRNA contents as crucial mediators of cell proliferation in VSM cells with a synthetic hyperproliferative phenotype. VSM cell proliferation is in part regulated by mitochondrial bioenergetics/ dynamics. Significantly, we have for the first time demonstrated a relationship between mitochondrial function, exosomal trafficking and VSM cell proliferation.

	Size (nm)	Protein (ug/250 ul)	RNA Yield (ng/ul)
Fresh W/T VSM cells	84.6 ± 0.8	120 ± 5	70.2 ± 10.2
21 day cultured VSM cells	66.2 ± 0.5*	130 ± 2.5*	118.7 ± 2.4*

Table 1. Exosomal size, protein and RNA content measured in exosomes isolated from freshly isolated WT (wild type) and 21- day cultured VSM cells. *p< 0.05 21 day cultured vs. WT.

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PC02

Drug Development For The Treatment Of Mitochondrial Optic Neuropathies

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In recent years, an increasing number of studies have shown evidence of mitochondrial involvement in a variety of neuro-ophthalmologic diseases.[1] Unfortunately, to date the treatment of mitochondrial disease is currently limited to symptomatic therapies, and there is no cure or substantial treatment option that guarantees a significant improvement and consequently, thus there is a pressing need to identify novel therapies. An approach that could represent a useful solution to treat these conditions is the identification of small molecules that restore energy production and/or reduce the level of oxidative stress. In the last few years, Idebenone has shown promising evidence of efficacy in limiting vision loss in patients with different mitochondrial dysfunctions.[2] Nevertheless, researchers have raised some concerns about idebenone, which can inhibit Complex I, due to a slow dissociation from the binding-site and increase the production of superoxide.[3] Furthermore, there is uncertainty around the mechanism of action of Idebenone, recently it was proposed that Idebenone activity lies on its metabolic activation from the NQO1 (NADH-Quinone oxidoreductase-1) enzyme and the reduced form of Idebenone (Idebenol) can act as electron donors for Complex III, thereby bypassing dysfunctional Complex I to provide cellular energy rescue as well as antioxidant activity.[4] In the light of this recent studies, the aim of this project is to investigate novel compounds that, targeting NQO1, show cellular energy rescue as well as antioxidant properties avoid Idebenone side effects. Given the abundance of crystal structure

information available for NQO1, an in silico approaches were applied to screen a library of commercial compounds. The selected compounds, were tested in specific in vitro assays, both in cell-free systems and in cells, in order to investigate their mode of action and their ability to enable mitochondrial respiration. In particular, the compounds were analysed to determine if they can act as electron acceptors from NQO1, and/or Complex I, an essential enzymes, which catalyse the first step of the mitochondrial electron transport chain.[5] The compounds were then tested in cellular assays using two immortalized cell lines, HepG2 and R28, to determine whether they can maintain cellular energy production by-passing the inhibition of Complex I. This evaluation was made by monitoring the compounds effect on reductive activity, with an MTT assay, and on ATP production, with a lucipherase assay. These initial findings indicate that the compounds identified in this study might be associated with a specific mechanism of action, involving a significant turnover by NQO1 corresponding to a significant increase of ATP production under Complex I impairment conditions Figure1 and Figure2. This evidence makes these compounds a promising starting point for further structure optimization.

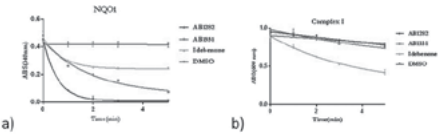

Figure 1 Results obtained for the Complex I assay and NQO1 assay. The graphs a and b illustrate the result obtained for Idebenone and analogs for the NQO1 and Complex I assay respectively. The assay was performed using 96 well and the following concentrations: 1µg/m NQO1, 120µM NADH, 3 mg/ml BSA, 300 µM KCN , 20µM Rotenone, 40-80 µM Compounds, 80µM DCPIP, Tris-Cl 25mM pH 7.4.The values are means ± SM, n=3 replicate wells, N=3.

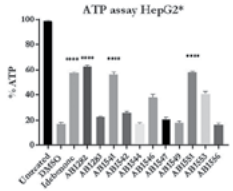

Figure 2 Results obtained for ATP assay. Compounds are tested at 1µM concentration. ATP rescue is defined as the percentage of quinone-induced increase in ATP levels in the presence of rotenone. The values are means ±SM, n=6 replicate wells, N=3.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC03

Serial Block Face Electron Microscopy Reveals Distinct Features of Mitochondrial Form in Striated Muscle

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Introduction

Mitochondria are important organelles for fuelling cellular function. They are known to have malleable morphologies that may relate to their involvement in pleiotropic cell functions and possibly mitochondrial-related diseases. However, analysis of the structure of mitochondria, and their positioning as a 'network', would benefit from extending examinations beyond traditional spatial resolutions of fluorescent confocal microscopy or single section transmission electron microscopy (TEM). With the development of Serial Block Face Scanning EM (SBFSEM), it is now possible to analyse and reconstruct mitochondria and surrounding elements in the x, y and z dimensions and at nm resolution. The purpose of this study was to investigate the range of whole mitochondrion morphologies evident in skeletal muscle cells.

Method

The psoas and soleus muscle from adult guinea pigs (killed according to Home Office guidelines) were collected. The samples were processed using a SBFSEM heavy metal protocol (Deerinck, 2010) and prepared for examination within the SBFSEM. The samples were imaged at a low magnification (2-4kx) with varying image size (2000-3000pixels) and resolution (8-19nm), and at least 250 serial sections (70nm) taken. The raw data was analysed manually in Microscopy Image Browser (MIB) and subsequent segmentations were reconstructed in 3D using Amira. This type of analysis is time consuming so only a portion of the visualised mitochondria was segmented. These were selected randomly by placing a grid (5 to 10μm2) over the series of images. Then each whole mitochondrion that crossed the grid, on a slice chosen randomly, was reconstructed.

Results

Visualisation of the raw data showed that mitochondria, both sub-sarcolemmal and inter-sarcomeric, could extend over 50 to 150 images, indicative of mitochondria having a range of volumes from 0.05 to 5.03μm3 (psoas) and 0.06 to 8.06 μm3(soleus). Several general morphologies were categorised as follows: columnar, spheroidal, framework or irregular (Figure 1). In addition, mitochondrial branching features were noted that varied in complexity from no branches, singular or

multiple segments to those of horseshoe or doughnut appearance. In psoas muscle cells there appeared to be more columnar mitochondria (71+12%) than in the soleus (17+14%, p=0.06, n=3, mean+sem, student's unpaired t-test), although it did not reach significance.

Conclusion

In summary, SBFSEM is a powerful technique for examining individual mitochondrion structure. We have found that there are distinguishable morphologies of mitochondria in skeletal muscle and a classification system has been developed to analyse these. These approaches will inform further studies investigating mitochondrial morphological changes with development and disease.

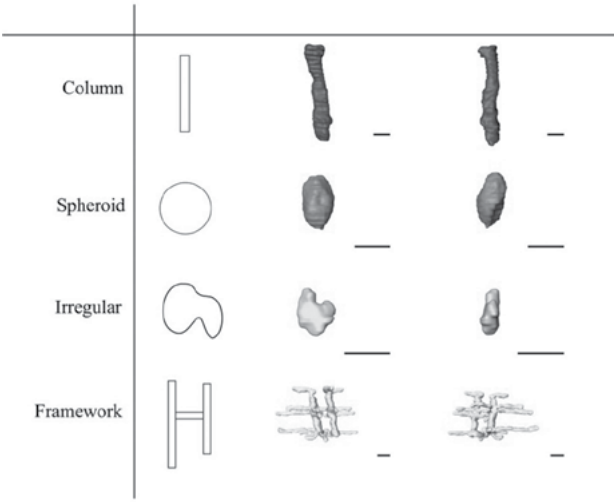

Figure 1. Mitochondrial Shape Classification. Shows examples of each of the basic morphologies of individual mitochondria observed in skeletal muscle. All Scale bars 1μm.

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PC04

Studying mitochondrial bioenergetics in ghrelin-mediated neuroprotection

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterised by progressive memory loss and cognitive decline. Calorie restriction (CR) is known to prevent cognitive deficits in mouse models of AD and in aged humans. Our group showed that CR-mediated neuroprotection, neurogenesis and memory are dependent on the hormone ghrelin, produced in the stomach in response to changes in the body's metabolic status. Acyl-ghrelin (AG) activates Growth Hormone Secretagogue Receptor-1a (GHSR) in several areas of the brain. In the arcuate nucleus of the hypothalamus, AG regulates food intake, adiposity and insulin secretion during CR, through AMP-activated kinase (AMPK). In the hippocampus, AG affects neurogenesis and synaptic density, increases long-term potentiation and enhances learning and memory[1,2].

Recent evidences showed that ghrelin activity involves mitochondrial signalling. Cells treated with serum from calorie-restricted rats, displayed reduced mitochondrial membrane potential (MMP) and ROS production, increased mitochondrial biogenesis and bioenergetics capacity[3]. Neurons pre-treated with ghrelin exhibit increased resistance to rotenone-induced toxicity and reduced cytocrome C release[4]. Increased ghrelin levels activate mitochondrial respiration, increase mitochondrial number, and contribute to fatty acids oxidation and ROS clearance[5]. Preliminary data from our group, in *in-vitro* and *in-vivo* models of Parkinson's Disease, showed that AG attenuates rotenone-induced dopamine neurone loss in nutrient restricted media; induces phosphorylation of AMPK and Acetyl-CoA carboxylase, a fatty acid biosynthesis regulator; promotes MMP and partially prevents rotenone-induced mitochondrial fragmentation (unpublished).

Our hypothesis is that AG-mediated neuroprotection may occur though regulation of mitochondrial energetic metabolism and fusion/fission balance. To investigate this, human neural stem cells (*ReN VM*, Merk Millipore) will be differentiated to mature neurones and treated for 5 days with AG before being incubated for 24h with amyloid-beta(1-42)oligomers (AβOs) - a peptide known to induce neuronal toxicity and mitochondrial dysfunction in AD patients[1]. To assess mitochondrial respiration and bioenergetics we will use an *Agilent Seahorse XF* system and MMP and mitochondrial fusion/fission will be quantified using confocal microscopy (*HCS Mitohealth* assay, Life Tech). Finally, using post-mortem human brain tissue (Brains for Dementia Research), we will determine GHSR expression in hippocampus and entorhinal cortex in AD and whether this correlates with expression of mitochondrial proteins.

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PC05

Identifying Novel Otoprotectants and Investigating their Mechanism of Action

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Background - The aminoglycosides (AGs) are a class of broad-spectrum antibiotic widely prescribed to treat life-threatening bacterial infections such as septicaemia. Whilst the AGs are highly efficacious, they are ototoxic. The AGs enter sensory hair cells of the inner ear through their mechano-electrical transducer (MET) channels. Once inside the cell they are thought to trigger mitochondrial dysfunction, thereby initiating apoptosis. To identify potential otoprotectants that might prevent AG-induced ototoxicity I have conducted a screen of the Tocris ion channel library.
Methods - Two assays were used, whereby gentamicin (a commonly-prescribed AG) was applied with or without the compounds for the duration of the experiment: 1) a 6-hour assay monitoring the death of zebrafish lateral line hair cells and 2) a 48-hour assay to assess the death of hair cells in mouse cochlear cultures. Electrophysiological recordings were subsequently carried out, examining ionic currents from mouse cochlear hair cells, to evaluate potential mechanisms of protection. Furthermore, an assay using isolated rat liver mitochondria was used to assess the effect that gentamicin may have on respiratory activities and whether any of the identified protectants work by combating this effect.
Results - Of the 160 compounds tested, 13 consistently protected mouse cochlear hair cells from 5 μM gentamicin when tested at a concentration of 50 μM. These 13 compounds were re-screened at a higher concentration (100 μM) in the absence of gentamicin. Three of the 13 were without obvious toxic side effects and of these, all three protected at 10 μM, two at 500 nM, and only one (XE 991) at 10 nM. These three compounds were also protective at 100 μM against 10 μM gentamicin in the zebrafish assay.
Electrophysiological recordings revealed that only one of the compounds blocks the MET channels whereas all three block the basolateral potassium channels ($I_{K,neo}$) to varying degrees. For XE 991, which does not block the MET channel at 50 μM, the modest level of $I_{K,neo}$ block (~40% at 30 μM) argues against depolarisation as the mechanism of protection.
Mitochondrial investigation shows that gentamicin stimulates state four (ADP-limited) and inhibits state three (ADP-dependent) respiration. Preliminary

experiments show that the second most successful Tocris compound combats this effect, preventing the gentamicin-induced stimulation of state four respiration.
Conclusions - Our screen of known ion channel blockers has identified compounds that protect hair cells from AG-induced death *in vitro,* with multiple mechanisms of action. One of these, XE 991, protects hair cells at nanomolar concentrations and may prove to be a suitable lead compound for the development of a clinically viable otoprotectant.

Sussex University

Medical Research Council

Action on Hearing Loss

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PC06

Regulation of mitochondria by ARMCX proteins in retinal pigment epithelial cells

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Situated at the back of the retina, the retinal pigment epithelium (RPE) is a dynamic tissue, which is essential for visual function. It is arguably the most metabolically active tissue in the human body and co-ordinates numerous processes to aid photoreceptor function, including phagocytosis of outer segments, ion transport, light absorption and photo pigment recycling. It also protects the neuroretina by acting as physical barrier and regulating oxidative stress. RPE mitochondria are situated in highly organised basolateral membrane infolds in close apposition to the choroidal blood supply. Their number and organisation decreases with age, and this is significantly worsened in age-related macular degeneration (AMD)[1]. Indeed, mitochondrial dysfunction has been implicated in the pathogenesis of AMD, where gene expression analysis indicates that expression of ARMCX3, a regulator of mitochondrial trafficking in neurons[2], is down-regulated in the early stages of the disease[3]. All six gene products of the ARMCX family are specific to Eutherian mammals and, based on finding from ARMCX3, are thought to be involved in mitochondrial trafficking and dynamics[2]. Differentiation of the RPE causes an up-regulation of ARMCX expression[4], indicative of an important role in the mature tissue.
We hypothesised that ARMCX proteins are key to RPE function by regulating mitochondrial function, in particular their dynamics. To this end, we have overexpressed GFP-tagged ARMCX1-3 and ARMCX5-6 in cultured RPE cells and found that all ARMCXs except ARMCX5 co-localised with mitochondria. Co-localisation was also confirmed for endogenous ARMCX by indirect immunostaining. Time-lapse

fluorescence microscopy revealed that overexpression of ARMCX1-3 or ARMCX6 led to collapse of mitochondrial networks and induced reduced trafficking. These results provide evidence of ARMCX proteins as regulators of mitochondrial dynamics in the RPE, and suggest that down-regulation of ARMCX may contribute to the pathogenesis of AMD, through dysregulation of the mitochondrial network.

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PC07

Defective metabolism provokes delayed hormone secretion in pancreatic islets

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Aim. Oxidative metabolism of pancreatic β-cell couples increases in peripheral blood glucose to secretion of hyperglycaemic hormone insulin. In prediabetic condition, insulin secretion is elevated and, interestingly, the hormone is secreted even after the removal of glucose stimulus, i.e. under the *de facto* basal conditions. Higher rates of basal, non-stimulated insulin secretion are reported in pancreatic islets isolated from type 2 diabetic (T2D) individuals, which can be modelled in islets from healthy donors by chronic hyperglycaemia. We aimed to elucidate the mechanism of the delayed response and the role of mitochondrial metabolism in this phenomenon.

Materials and Methods. Experiments were performed on isolated mouse and human pancreatic islets. The dynamics of intracellular concentrations of ATP, Ca^{2+}, cAMP, NAD(P)H, ψ_m and DAG was monitored in the islets cells in response to metabolic stimuli using laser scanning confocal or zoom microscopy. Insulin secretion was assayed using ELISA.

Results. Chronic hyperglycaemia resulted in an extended acute metabolic response. Intracellular Ca^{2+} dynamics was observable in islets pre-cultured at high glucose for up to 1h after the return into basal glucose, which was the result of a rapid and prolonged increase of intracellular ATP/ADP ratio in response to the removal of

the glucose clamp. This ATP "off-response" depended on the duration of the acute exposure to high glucose; it was observable in the case when methyl-succinate was used as a metabolic stimulus and was reflected in insulin secretion kinetics. Chronic hypoglycaemia, introduction of creatine in the culture medium, or genetic knock-out of fumarate hydratase (Fh1βKO) significantly attenuated the effect. The off-response was absent in the kinetics of DAG, which is synthesised *de novo* from glucose/glycerol-3-phosphate in β-cells, in a malonyl-CoA-dependent manner.
Conclusions. The mechanism responsible for the elevated basal insulin secretion in prediabetic pancreatic islets relies on the prolonged/inert transduction of the metabolic stimulus in β-cells. The phenomenon has mostly a mitochondrial nature. The lack of the effect in the DAG kinetics argues against the anaplerotic origin and the ability of methylated succinate to induce the off-response excludes any glycolytic/glycogenolytic options too. The source of the signal can be restricted to the set of the TCA cycle reactions from aconitase to α-ketoglutarate as fumarate abundant in the Fh1βKO animals is likely to inhibit the former enzyme.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC08

Dimethylfumarate as a treatment for multiple sclerosis: a role for mitochondria?

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Mitochondria have been recently recognized as a key player in the pathophysiology of multiple sclerosis (MS), and mitochondrial membrane potential ($\Delta\Psi_m$) is decreased in animals with experimental autoimmune encephalomyelitis (EAE), a model of MS. Dimethylfumarate (DMF), a treatment for multiple sclerosis, is thought to act on inflammation and oxidative stress, specifically via upregulation of the transcriptional factor Nrf2. As mitochondrial metabolism may be regulated by Nrf2, and fumarate participates in the Krebs cycle, we have explored whether DMF might protect $\Delta\Psi_m$ in EAE.
Methods: Female mice with transgenically labelled axonal mitochondria were immunized with MOG_{35-55} and treated prophylactically with DMF (30mg/kg; twice daily) or vehicle. At eight days post-immunisation, and on the first day of neurological deficit, the spinal cord of the mice was exposed for *in vivo* confocal imaging of $\Delta\Psi_m$, under terminal anesthesia, using the fluorescent dye TMRM. Subsequent immunohistochemical examination included assessment of inflammation and Nrf2 upregulation. Complex I and II activity was analyzed in spinal cord mitochondrial isolates from immunized mice to determine a deficit in their enzymatic activity.
Results: Prophylactic DMF treatment significantly reduced EAE incidence, but did not affect disease course. The decrease in $\Delta\Psi_m$, normally observed in axonal

mitochondria shortly before the expected onset of neurological deficit, was averted in the DMF-treated animals, whereas after disease onset $\Delta\Psi_m$ was decreased in both treatment groups. Post mortem examination showed significantly increased levels of Nrf2 in the DMF-treated animals, within 8 days of treatment. Inflammatory cell infiltration was minimal at the preclinical stage of the disease for both treatment groups, and microglial activation was not affected by DMF treatment. Topical application of DMF had no significant effect on $\Delta\Psi_m$ compared with vehicle-treated mice.

Conclusions: In the days preceding the onset of neurological deficit, DMF treatment results in the preservation of $\Delta\Psi_m$ of axonal mitochondria, which can be the underlying mechanisms of the observed decrease in EAE incidence compared to controls. Although acute application of DMF is not sufficient to avert the mitochondrial deficit, Nrf2 upregulation following systemic DMF upregulation may play a role in the observed mitochondrial protection. $\Delta\Psi_m$ preservation does not seem to occur via an Nrf2-mediated decrease in inflammation, as shown by the minimal presence of infiltrating inflammatory cells, as well as the lack of an effect on the levels of activated microglia following DMF treatment.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC09

Tetracycline antibiotics impair cardiac mitochondrial and contractile function

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Tetracyclines antibiotics act through inhibiting bacterial protein translation. Given the bacterial ancestry of mitochondria, we tested the hypothesis that doxycycline reduces mitochondrial function, and results in cardiac contractile dysfunction. We cultured H9C2 cardiomyoblasts and adult rat cardiomyocytes (male, 250 g, n=3; anaesthetised with isoflurane) in the presence of doxycycline (0, 10 and 30 μg/ml, DOX0, DOX10 and DOX30 respectively). Ampicillin and carbenicillin (both 100 μg/ml) were used as control antibiotics. ANOVA was performed to assess differences between groups and values are presented as mean±stdev. The ratio between protein abundance of a mtDNA-encoded OXPHOS subunit (cytochrome c oxidase subunit I) and a nDNA-encoded OXPHOS subunit (Succinate DeHydrogenase A) was 39±5% lower in DOX30 compared to control (p=0.02), with intermediate values for DOX10, confirming a mitonuclear protein imbalance in doxycycline-treated H9C2 cells. Maximal uncoupled respiration was lower in DOX30 than control (136±22 *vs.* 272±25 pmol.μgDNA^{-1}.min^{-1}, p=0.01). Complex I respiration (-66±20%) was more affected compared to complex II respiration (-46±36%) in DOX30. Interestingly, also the protein content of the nuclear-encoded complex I subunit NDUFS3 was

significantly reduced by ~50% in DOX10 and DOX30, indicative of a mitonuclear imbalance that goes beyond a reduced mitochondrial protein synthesis in doxycycline-treated H9C2 cells. TMRM-loaded mitochondria (200 nM) appeared fragmented and the content of mitochondrial fission-related Dynamin related protein 1 (DRP1) was 43±9% larger in DOX30 compared to DOX0. Cardiac contractility was assessed by Fura-2AM in electrically stimulated (at 0.5 Hz) adult rat cardiomyocytes kept in culture for 2 days. Diastolic calcium concentration was significantly higher in DOX10 (0.68±0.01) *vs.* DOX0 (0.64±0.01, p=0.01). No differences were observed in other parameters of the calcium transient. We conclude that tetracycline antibiotics impair mitochondrial function, particularly mitochondrial complex I respiration, cause fragmentation and result in diastolic dysfunction in adult cardiomyocytes. These results could have major implications for giving specific classes of antibiotics to patients at risk of developing cardiovascular dysfunction. Also, scientists should be aware of the potential confounding effects of certain classes of antibiotics (including streptomycin) on experimental results.

This work was funded by a grant of the Amsterdam Cardiovascular Sciences to RCIW and RHH.

I confirm that the above ethical and content criteria have been met and understand that The Society reserves the right to reject the abstract should it not conform to the above guidelines.

PC10

Mitochondria-targeting hydrogen sulfide donors prolong healthspan: lifespan ratio in Caenorhabditis elegans

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Progressive muscle atrophy is characteristic of several chronic debilitating conditions, including ageing (sarcopenia), muscular dystrophies, diabetes, bedrest and spaceflight. Whilst the precise mechanisms of slow atrophy are poorly defined and multifactorial, impaired mitochondrial 'function' (e.g. oxidative capacity and fusion-fission dynamics) is a common feature and represents an attractive target for therapy. Nonetheless, effective countermeasures remain elusive. Hydrogen sulfide (H_2S) is an endogenous 'gasotransmitter' with important roles in several biochemical processes, including the maintenance of mitochondrial integrity, and in models of ageing 'H_2S bioavailability' is significantly reduced. Using *Caenorhabditis elegans* as an established model for muscle ageing, we have examined the role of a novel class of H_2S donors for promoting healthspan and lifespan. Unlike general non-targeted H_2S donor compounds with established efficacy in extending lifespan (e.g. GYY4137), we have examined compounds that drive targeted H_2S directly to the mitochondria by coupling H_2S-generating moieties

to a triphenylphosphonium motif (AP39) or mitochondria-targeting peptide sequences (RTP10). Our study shows that these compounds effectively preserve mitochondrial structure versus non-targeted H_2S donors (mitochondria::GFP fragmentation: AP39 = ≥10 d, GYY4137 = 6 d post-adulthood). Mitochondrial H_2S also improved animal movement rate (movement across the lifespan (mean ± SEM): AP39 = 73.2 ± 9.6, GYY4137 = 57.6 ± 27.6 strokes.min^{-1}, P <0.01) and extended lifespan (median survival: AP39 = 10 d; GYY4137 = 8 d post-adulthood, P < 0.001). Importantly, these compounds were effective at concentrations orders of magnitude lower than traditional H_2S donors (e.g. ≤ 100 nM vs.≥50 mM). Our study strongly suggests that enhancing mitochondrial function *via* exogenous mitochondria-targeting H_2S might be an effective treatment strategy for preserving muscle health during ageing for improving the healthspan: lifespan ratio. Mitochondrial H_2S supplementation may also hold future efficacy for other muscle mitochondrial pathologies.

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PC11

Development of Sorafenib resistance in hepatocellular carcinoma is mediated by SIRT1

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Sorafenib is a multi-kinase inhibitor as well as an inhibitor of oxidative phosphorylation (OXPHOS) (Fiume et al. 2011) and frequently the only treatment option for patients with non-resectable hepatocellular carcinomas (HCCs). Resistance to Sorafenib develops frequently and could be mediated by the NAD dependent deacetylase sirtuin (SIRT) 1 (Chen et al. 2012), a master regulator of cellular energy metabolism and stress responses. SIRT1 activity is regulated by cellular NAD+ concentrations and NAD salvage by nicotinamide phosphoribosyltransferase (NAMPT) (Revollo et al. 2004). We aimed to find out if Sorafenib treatment induces changes in cellular NAD levels and activity of Sirt1 and the cellular energy sensor adenosine monophosphate kinase (AMPK) and whether or not altering Sirt1 activity by overexpression or increasing cellular NAD levels could influence Sorafenib action on HCC in vitro.

We could show that Sorafenib treatment of HCC cell lines (Huh7, Hep3B, HepG2) induced apoptosis 2-fold in Hep3B and HepG2 and 5-fold in Huh7 at 5 uM Sorafenib. Oxygen flow measured by high resolution respirometry in permeabilised cells was lower after Sorafenib treatment (1 and 5 uM for 24h) both in the leak (non-phosphorylating) and OXPHOS state. The ratio of H_2O_2/O as as measure for reactive oxygen species (ROS) production normalized to oxygen flow was increased

after Sorafenib (1 uM) exposure for 24h, specifically in the leak state and after inhibiting complex III of the electron transport chain with Antimycin A.
SIRT1 protein was downregulated and cellular NAD concentrations were significantly lower in Huh7 cells after exposure to Sorafenib for 24h. Concomitant to increasing phosphorylation of AMP kinase, activity of its downstream target mammalian target of rapamycin (mTOR) was decreased after Sorafenib treatment, which could indicate energy deprivation consistent with the decreased mitochondrial respiration. Both number of apoptotic cells and effects on AMPK/mTOR phosphorylation were reversed by supplementation of nicotinamide mononucleotide (NMN), the enzyme product of NAMPT. While pharmacologically inhibiting NAMPT by FK866 did not sensitise HCC to Sorafenib treatment, transient overexpression of Sirt1 decreased Sorafenib-induced apoptosis.
We can therefore conclude that Sorafenib influences SIRT1 function and that overexpression of SIRT1 could be an underlying mechanism of resistance to Sorafenib treatment in HCC.

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PC12

Reduction in the vitamin D receptor results in impaired mitochondrial function in C2C12 Myoblasts

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Approximately 25% of the world's population is affected by vitamin D (VitD) deficiency. It has been reported to play a role in maintaining skeletal muscle function. Large scale epidemiological studies have established a link between VitD deficiency and sarcopenia [1]. The biological functions of VitD and its active metabolites are mediated by the VitD Receptor (VDR) and its control over nuclear transcription. The treatment of cholecalciferol in VitD deficient humans resulted in improvements in skeletal muscle phosphocreatine recovery suggesting VitD or its metabolites alter skeletal muscle oxidative capacity [2]. Despite this little is known about the precise role of the VDR in mediating these adaptations. Following the knock down of the VDR (VDR-KD) in the myogenic C2C12 cell line mitochondrial function was

assessed using the Seahorse XFe24 extracellular flux analyser. Scramble control and VDR-KD cells were plated at 30,000 cells/well (n=10). Following assays cells were lysed and data normalised to protein content. Values are means ± SD., and compared by T.Test. VDR-KD resulted in impaired rates of both basal (6.93 ± 1.02 vs. 9.86 ± 3.42 pmol/min/mg, p<0.05) and maximal respiration (19.65 ± 1.24 vs. 30.76 ± 10.42 pmol/min/mg, p<0.05) compared to scramble control. Further analysis revealed a reduction in total ATP production (45.0 ± 3.0 vs. 55.2 ± 8.1 pmolATP/min/mg, p<0.05) as well as a reduction in the contribution from oxidative phosphorylation (39.9 ± 3.5 vs. 46.1 ± 8.4 pmolATP/min/mg, p<0.05) in VDR-KD compared to scramble control. These data highlight an important role for the VDR in maintaining skeletal muscle mitochondrial function, specifically that related to oxidative metabolism.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC13

Title: Mitochondrial oxygen consumption a new target for paracetamol?

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Paracetamol has been known for some time to reduce core body temperature of both febrile and non-febrile animals by a mechanism that is not fully understood. Historically the actions of paracetamol were attributed to the inhibition of central cyclo-oxygenase enzymes however given the weak inhibitory effects on COX-1 and COX-2 enzymes alternative targets have been sought including a possible novel COX-3.

Both reduction of pyresis and hypothermia occurs only when metabolic rate (heat production) decreases and/or heat loss increases. In an attempt to determine if the hypothermic properties of paracetamol could be attributed to the impact on mitochondrial energy metabolism, studies were undertaken to assess the effect of paracetamol and two COX-3 inhibitors aminophenazone and phenazone on mitochondrial oxygen consumption in both 3T3-L1 mouse adipocytes and isolated mitochondria.

3T3-L1 adipocytes were seeded in XFp microplates and oxygen consumption rate (OCR) was measured using Seahorse XFp Analyser. At concentrations (5-10mM) known to cause hypothermia and antipyresis paracetamol, aminophenazone and phenazone cause a significant (up to 50%) inhibition of oxygen consumption in 3T3-L1 cells without affecting cell viability. Isolated rat liver mitochondria were plated in XFp microplates and then transferred to the Seahorse XFp Analyser. Using electron flow assay, paracetamol (10mM) was also able to inhibit oxygen consumption in a

manner similar to known inhibitors of the electron transport chain rotenone and antimycin A.
Both the maintenance of normal body temperature and the induction of pyresis require increased mitochondrial electron transport chain activity and associated oxygen consumption. The failure to find a central target for the hypothermic effects of paracetamol and ability of paracetamol and other hypothermic agents to inhibit oxygen consumption without a loss of cellular viability suggest that disruption of mitochondrial oxygen consumption observed in differentiated adipocytes and isolated mitochondria could partly explain the hypothermic properties of paracetamol and similar compounds.

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PC14

Oscillations in mitochondrial ROS production during the early cell cycles in Xenopus embryos

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The ability to repair and regenerate tissues is an essential process for the survivability and development of the organisms. Amphibians excel on these processes and are invaluable models to study the molecular and cellular mechanisms underlying scar free wound healing and tissue regeneration. Among these, we have used the frog, *Xenopus,* as an animal model to study the role of reactive oxygen species (ROS) during the early embryonic development and appendage regeneration. Both embryonic development and tissue repair/regeneration require cell proliferation, which relies on the synchronized mechanisms that regulate the cell cycle [1]. The mitochondrion is the powerhouse of the cell but it is also involved in other processes such as cellular signaling and calcium buffering. However, the roles of $_{mt}$ROS during early vertebrate development have remained largely unknown. For this reason, our main aim is to understand how the mitochondria, metabolism and ROS are regulated during early development and tissue regeneration. We have recently shown using transgenic *Xenopus* frog embryos expressing the genetically encoded ROS indicator HyPer that $_{mt}$ROS is increased after fertilization and that it oscillates during each cell division. When we exposed the embryos to mitochondrial inhibitors we observed that complex II is the primary source of ROS *in vivo* and that the inhibitors differentially affect the oscillatory patterns of ROS production. Furthermore, in order to identify the source of $_{mt}$ROS in the electron transfer system, we performed a study of mitochondrial function in a cell-free system (i.e. egg extract) combining high-resolution respirometry, hydrogen peroxide production and membrane potential [2]. Our study reveals that the succinate dehydrogenase complex (CII), specifically the flavoprotein in the SdhA subunit,

is the major source of $_{mt}$ROS when the mitochondria are fuelled by succinate. Also, we have found that the calcium acts upstream of ROS production from the mitochondria. Finally, we have discovered that the ATP levels also oscillate during the cell cycle and our results suggest that cellular metabolism alternates between aerobic glycolysis (Warburg Effect) and OXPHOS in association with the cell cycle. Our results highlight an entanglement between calcium, metabolism and ROS but further work is required to understand how these processes are related to the cell cycle and its relevance for the early development and tissue regeneration.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC15

Effects of type 1 and type 2 diabetes on the structure and pattern of distribution of ventricular mitochondria in the rat heart

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Diabetes mellitus (DM) is associated with hyperglycaemia (HG). Chronic HG induces oxidative stress and glycation of intracellular proteins (1). These events result in abnormal conformational and structural changes in the organelles of cardiomyocytes. One of the most sensitive organelles to respond to these changes is the mitochondrion. The ultrastructure and the pattern of distribution of mitochondria in streptozotocin (STZ)-induced diabetic rat, a model of type 1 and in the Goto-Kakizaki (GK) rat, a model of type 2 DM was investigated using electron microscopy (EM). The study was performed on 3-month and 18-month old STZ-induced diabetic rats and age-matched Wistar controls. The structure and pattern of distribution of mitochondria was also investigated in GK rats for comparison. Left ventricular tissue samples from diabetic and control groups were fixed in Karnovsky solution, dehydrated in ethanol, and embedded in resin. Ultrathin sections were cut with diamond knife, counterstained with uranyl acetate and lead citrate and viewed with Philips EM. The results showed a significant (Student's *t*-test; $p<0.05$) reduction in the number of mitochondria. In addition, the size of mitochondria is significantly ($p<0.05$) reduced in cardiomyocytes of STZ-induced and GK diabetic rats compared to control. This study has demonstrated severe abnormality in the structure and pattern of distribution of mitochondria in cardiomyocytes of both STZ-induced and GK diabetic rats when compared to controls. These mitochondrial anomalies may underlie the functional dysfunction observed in diabetic patients.

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PC16

Inhibition of Drp1 in the Dorsal Vagal Complex of the Brain Reduces Food Intake in Insulin Resistant Rats

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Worldwide obesity has more than doubled since 1980, with over 600 million cases in 2014. Obesity can lead to many adverse metabolic effects of the cardiovascular, brain and endocrine systems. In rodents, the dorsal vagal complex (DVC) of the brain regulates glucose homeostasis and controls food intake through insulin signalling. A 3-day high fat diet (HFD) has shown to induce insulin resistance thus diminishing the DVC's ability to regulate glucose metabolism and food intake, though exact mechanistic effects of this are still unknown. HFD feeding is associated with an increase in mitochondrial fission in the DVC. Mitochondrial fission is regulated by dynamin related protein 1 (Drp1), high levels of Drp1 have been shown to correlate with increased levels of inducible nitric oxide synthase (iNOS), increased endoplasmic reticulum (ER) stress and insulin resistance in the DVC. In HFD fed rodents, molecular and chemical inhibition of Drp1 significantly improves the ability of DVC to regulate glucose metabolism. Whether increased mitochondria fission in the DVC of HFD fed rats affect food intake is still not known. Our data indicates that pharmacological inhibition of Drp1 with MIDVI-1 in the DVC reduced food intake in HFD fed rats as early as one day of injection. This data suggests that decreasing mitochondria fission in the DVC is sufficient to reduce hyperphagia in HFD fed rats.

I confirm that the above ethical and content criteria have been met and understand that The Society reserves the right to reject the abstract should it not conform to the above guidelines.

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