QMB Mitochondrial Biology Abstract Book

M1: Assembly of a complex complex

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Complex I (NADH:ubiquinone oxidoreductase) is the first enzyme of the mitochondrial respiratory chain and is composed of 45 subunits in humans, making it one of the largest known multi-subunit membrane protein complexes. The enzyme is a boot-shaped structure of ~1MDa with a hydrophilic matrix arm and a hydrophobic membrane arm. These arms are assembled via a series of intermediate modules containing core and accessory subunits and stabilised by the transient association of different assembly factors. Mutations in genes encoding complex I subunits and assembly cause mitochondrial disease. The assembly of this complex machine will be discussed.

M2: Deletions and gene conversions in the *ATAD3* gene cluster result in cerebellar developmental defects linked to mtDNA and cholesterol

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Mitochondrial disorders are clinically heterogeneous with causative mutations identified in >260 genes. However, the molecular diagnosis is unknown in ~40% of cases, partly due to some genomic regions being refractory to analysis. Using SNP microarray and/or whole exome sequencing, we identified 6 subjects from 5 unrelated families with impaired cerebellar development and neurological function who carried deletions or gene conversions in the *ATAD3* locus (1). This locus consists of 3 highly homologous tandemly arrayed genes (*ATAD3C*, *ATAD3B* and *ATAD3A*) encoding mitochondrial proteins implicated in processes including mtDNA metabolism, protein translation and mitochondrial dynamics. Due to its complexity, rearrangements of the *ATAD3* locus have not been reliably detected by standard genomic analyses.

Five of the subjects had highly similar clinical presentations including congenital pontocerebellar hypoplasia and early death. In these subjects, 2 similar ~38 Kbp deletions resulted in an *ATAD3B*/*ATAD3A* fusion that produced an mRNA encoding a protein 99% identical to *ATAD3A*, but under the control of the *ATAD3B* promoter. This caused a striking reduction of ATAD3 in cells and tissues.

The 6th adult subject had milder features that included cerebellar atrophy and ataxia. The *ATAD3* rearrangements were less well resolved, but appeared to include a heterozygous deletion involving *ATAD3C*/*ATAD3B*, as well as gene conversion events in which segments of *ATAD3B* or *ATAD3A* were replaced by the other. In fibroblasts, this led to strongly decreased ATAD3B and reduced ATAD3A.

Multiple other subjects with distinct neurological syndromes were recently identified to have *de novo* dominant or homozygous recessive mutations in *ATAD3A* (2, 3). Together with our families, they present a broad clinical spectrum of disorders resulting from *ATAD3* rearrangements/mutations. Moreover, investigations in *ATAD3* patient fibroblasts revealed mtDNA abnormalities and indications of perturbed cholesterol metabolism. This integration of mitochondria and ATAD3 in cellular cholesterol homeostasis thus provides a molecular mechanism for the disease.

1. Desai, R., A.E. Frazier, et al. (2017) *ATAD3 gene cluster deletions cause cerebellar dysfunction associated with altered mitochondrial DNA and cholesterol metabolism*. Brain 140: 1595-1610.
2. Harel, T., W.H. Yoon, et al. (2016) *Recurrent De Novo and Biallelic Variation of ATAD3A, Encoding a Mitochondrial Membrane Protein, Results in Distinct Neurological Syndromes*. Am. J. Hum. Gen. 99: 831-845.
3. Cooper, H.M, Y. Yang, et al. (2017) *ATPase-deficient mitochondrial inner membrane protein ATAD3A disturbs mitochondrial dynamics in dominant hereditary spastic paraplegia*. Hum. Mol. Genet. 26: 1432-1443.

M3: Diagnosis of mitochondrial diseases

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Mitochondrial diseases are a diverse group of inherited disorders that affect mitochondrial function and compromise cellular homeostasis. They can exhibit exceptional clinical complexity and variability, which when combined with a lack of reliable biomarkers makes diagnosis a clinical challenge. We aimed to improve the diagnostic indication of mitochondrial diseases using a multivariate metabolic phenotyping approach.

We measured small metabolite profiles from control and patient-derived biofluids using Nuclear Magnetic Resonance spectroscopy. Identifiable metabolite peaks were used to create a multivariate disease discriminant model. The model was then tested on validation cohorts to determine the diagnostic sensitivity of the model.

The multivariate metabolic model outperformed historical markers creatine kinase, lactate, pyruvate and the lactate:pyruvate ratio, as well as the recently identified single serum markers Fibroblast Growth Factor 21 and Growth Differentiation Factor 15. Inclusion of the single serum markers in the multivariate model increased the diagnostic sensitivity further. This indicates that metabolic phenotyping of patient biofluids may be a reliable means of indicating mitochondrial diseases in order to triage patients to subsequent comprehensive and definitive diagnosis.

M4: Blockade of the mitochondrial transition pore in diabetic kidney disease

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Diabetes Mellitus, including both type 1 (T1D) and type 2 (T2D), is one the most important global health issues of the 21st century. Approximately 30% of patients will develop diabetic kidney disease (DKD). Few effective therapies are available to slow the clinical progression of DKD to End Stage Renal Disease; which requires dialysis or renal replacement therapy. Much of the underlying aetiology of DKD remains unknown, however mitochondrial dysfunction is co-associated with the disease. Studies from our laboratory and others have shown a decline in mitochondrial function in the kidney in DKD, leading to ATP depletion, changes in mitochondrial morphology and renal fibrosis. In addition, a greater susceptibility to mitochondrial permeability transition (mPT) pore opening is observed. In this study, we determined whether reducing mPT pore capacity would ameliorate diabetes-induced renal injury using both gene knockout and pharmacological strategies to directly interrupt mPT pore function. In study 1, we used mice with a genetic knockout of the functional unit of the pore, Cyclophilin D *(Ppif -/-*). Wild type and *Ppif -/-* mice (n=15/group) were rendered diabetic using streptozotocin (55mg/kg/day, a model of T1D) and followed for 20 weeks. In study 2, we used a mouse model of T2D, the db/db mouse. Db/db mice and their littermate controls (db/h) were randomized to receive a Cyclophilin D inhibitor, the non-immunosuppressive Cyclosporin A analogue, Debio-025, by daily oral gavage (10mg/kg/day) for 16 weeks. Glycemic control was unchanged for both the Diabetic *Ppif -/-* and *Ppif WT* STZ mice. Likewise, Debio-025 did not alter glycemic control in db/db mice. Preliminary data show that neither the genetic deletion of cyclophilin D, nor its therapeutic targeting using Debio-025 reduced renal injury (observed by no change in albuminuria). This study suggests that the mPT pore is not a suitable therapeutic target in DKD.

M5: Insights into the prognostic association of fatty acid oxidation in cancer

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Alterations in cellular metabolism and energetic state are a hallmark of cancer cells. Many tumours rely heavily on aerobic glycolysis for their metabolic needs, and are less dependent on mitochondrial respiration. However, the association between metabolic pathways used in tumours and cancer survival rates remains poorly defined. Fatty acid oxidation (FAO) is an important metabolic pathway that occurs in the mitochondrial matrix, and its relevance in cancer biology is contentious.

In this study, we utilised computational and experimental methods to identify and characterise the prognostic association between FAO and patient outcome in several tumour types, with a particular focus on breast cancer. We observed that patients with high expression of a gene signature involved in FAO have significantly better outcome than patients with low expression. Furthermore, in 11 different tumour types, we observed consistent downregulation of the FAO signature expression in primary tumours, compared to normal tissues, suggesting that reprogramming of FAO may occur during tumourigenesis. Of note, we found that the expression of the FAO signature expression could predict response to pre-surgical endocrine or chemotherapy in breast cancer patients.

We generated cell systems with overexpression and knockdown of carnitine palmitoyl transferase 1A (*CPT1A*) – the rate-limiting enzyme in FAO. These systems were characterised using qPCR, immunoblotting, metabolic flux analysis in response to exogenous palmitate using the Seahorse XF technology. Furthermore, we observed that overexpression of CPT1A in the MDA-MB231 cell line significantly decreased proliferation and wound healing migration rates, compared to the basal expression control. However, no significant difference in anchorage-independent growth was observed between basal and CPT1A overexpression in MDA-MB231 cells.

To summarise, we have identified a gene signature involved in FAO to be prognostic in multiple cancers. Our findings suggest the use of agents that can upregulate FAO may be efficacious in the treatment of some cancers.

M6: Insights into function from naturally occurring cytochrome *c* mutations

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Cytochrome *c* has crucial roles in cell life and death. Naturally occurring cytochrome *c* mutations cause autosomal dominant thrombocytopenia (low platelets)[1]. The molecular basis of the platelet release abnormality is unknown, however it does not appear to be due to a defect in the electron transport function of cytochrome *c* in the respiratory chain. Instead mutation of cytochrome *c* alters two cytochrome *c* functions implicated in apoptosis – caspase activation and peroxidase activity[1-3]. It has been proposed that cytochrome *c*-catalysed oxidation of the inner mitochondrial membrane lipid cardiolipin is required for released of proapoptotic factors, including cytochrome *c* itself, from mitochondria to the cytosol in response to activation of the intrinsic apoptosis pathway. However questions remain regarding the details of the peroxidase activity, and the exact role of cardiolipin oxidation in apoptosis, and whether peroxidase-active cytochrome *c* can trigger caspase activation.

Here I will describe our *in vitro* analyses of the apoptotic and peroxidase activities of new cytochrome *c* variants, which provide novel insights into the relationship between these different activities. Our current results indicate that the conversion of cytochrome *c* into an active peroxidase enzyme prevents cytochrome *c*-induced caspase activation, suggesting that these two activities are mutually exclusive. Further work is being undertaken in isolated mitochondria to determine the relationship between these two activities *in vivo*.

1. Morison, I. M., Cramer Bordé, E. M., Cheesman, E. J., Cheong, P. L., Holyoake, A. J., Fichelson, S., Weeks, R. J., Lo, A., Davies, S. M. K., Wilbanks, S. M., Fagerlund, R. D., Ludgate, M. W., da Silva Tatley, F. M., Coker, M. S. A., Bockett, N. A., Hughes, G., Pippig, D. A., Smith, M. P., Capron, C., and Ledgerwood, E. C. (2008) *A mutation of human cytochrome c enhances the intrinsic apoptotic pathway but causes only thrombocytopenia.* Nat Genet 40, 387–389.

2. Josephs, T. M., Liptak, M. D., Hughes, G., Lo, A., Smith, R. M., Wilbanks, S. M., Bren, K. L., and Ledgerwood, E. C. (2013) *Conformational change and human cytochrome c function: mutation of residue 41 modulates caspase activation and destabilizes Met-80 coordination*. J Biol Inorg Chem 18, 289–297.

3. Josephs, T. M., Morison, I. M., Day, C. L., Wilbanks, S. M., and Ledgerwood, E. C. (2014) *Enhancing the peroxidase activity of cytochrome c by mutation of residue 41: implications for the peroxidase mechanism and cytochrome c release.* Biochem J 458, 259–265.

M7: Exploring mitochondrial stress signalling

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Mitochondria are crucial organelles involved in energy transduction, biosynthesis of macromolecules and apoptosis. More recently it has emerged that mitochondria are also very active cell signalling platforms. Due to the endosymbiotic nature of mitochondria and as a by-product of energy transduction via the electron transport chain, these organelles are inherently prone to an array of different stresses including bioenergetic stress, oxidative insults, genomic damage and proteotoxic stress. The molecular pathways that are engaged by mitochondria to respond and overcome these stresses are not fully elucidated and in this presentation I will discuss some of our recent work exploring intracellular and extracellular signalling mechanisms induced by bioenergetic stress.

M8: The insulin receptor in ageing and fatty liver disease

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Impaired insulin/IGF1 signalling has been shown to extend lifespan in model organisms ranging from yeast to mammals, in part by enhancing mitochondrial oxidative capacity. Whereas hyperinsulinemia associated with elevated energy intake promotes lipogenesis, and can reduce mitochondrial capacity directly contributing to the development of a metabolic disease phenotype. In a series of studies we investigated how reducing insulin levels or insulin receptor expression an adult mice effects lifespan and susceptibility to diet induced metabolic disease. In conflict with findings in metazoans like *C. elegans* and *D. melanogaster*, our results suggest that the impairment of insulin signalling limited to peripheral tissues of adult mice fails to extend lifespan despite increased systemic insulin sensitivity and elevating mitochondrial capacity. However, reduced circulating insulin levels and partial reduction of the insulin receptor in peripheral tissues protects against the development of fatty liver disease. These findings suggest that treatments that enhance liver insulin signalling during conditions of metabolic overload may promote the development of non-alcoholic fatty liver disease. Furthermore, the lifespan extending effect of impaired insulin signalling in mammals may require reduced central nervous system insulin signalling or the impairment of insulin signalling during development, potentially limiting the effectiveness of this strategy to extend health-span of those who are already aged.

M9: Novel role for the mitochondrial sodium/calcium exchanger NCLX in regulating VEGF-induced signaling in endothelial cells

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Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. Important for this action of VEGF on endothelial cells (ECs) is the phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser^{1177} leading to eNOS activation and increased production of nitric oxide (NO). Increasing evidence indicates that mitochondria play key signaling roles in ECs, frequently involving the production of reactive oxygen species (ROS). In this study, we found that VEGF-induced eNOS activation in ECs required functional mitochondria. Thus, inhibition of the mitochondrial respiratory chain at complex I with rotenone or uncoupling the mitochondrial membrane potential with carbonylcyanide-m-chlorophenylhydrazone (CCCP) significantly attenuated VEGF-induced eNOS activation. Activation of eNOS also required a proximal signaling pathway involving VEGF receptor-2 (Flk-1)/Src kinase-dependent activation of phospholipase C (PLC)-induced intracellular calcium signaling. Mitochondria acted downstream of Flk-1 and PLC, as rotenone and CCCP did not significantly attenuate activation of these proteins in response to VEGF. Mitochondria's signaling role was independent of ROS and instead involved control of VEGF-induced calcium signaling. Thus, rotenone or CCCP inhibited VEGF-induced cytosolic calcium transients. Also, inhibition of the mitochondrial sodium/calcium exchanger NCLX with the pharmacological inhibitor CGP37157 or NCLX-targeted siRNA attenuated VEGF-induced cytosolic calcium transients and eNOS activation. Moreover, VEGF stimulated a rapid and transient increase in mitochondrial calcium that was significantly prolonged by NCLX inhibition with CGP37157 or NCLX-targeted siRNA. Together these findings identify a previously unrecognized role for mitochondrial NCLX in controlling intracellular calcium signaling leading to eNOS activation in VEGF-stimulated.

M10: Amino acid signalling in metabolic disease

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M11: (Re)Defining the molecular control of mitochondrial apoptosis

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BAX and BAK are the essential effector proteins that promote mitochondrial outer membrane permeabilisation and cell death during apoptosis. Understanding the protein interactions that control their deadly apoptotic activity will identify new ways to target apoptosis therapeutically. The mitochondrial anion channel VDAC2 is thought to be a critical inhibitor of the pro-apoptotic protein BAK, with hyperactive BAK proposed to drive lethality in *Vdac2*$^{-/-}$ mice. Our studies challenge this dogma as *Vdac2*$^{-/-}$ mice survive embryonic development without the need to delete *Bak*. The mice exhibited no evidence of excessive BAK-mediated apoptosis, but die prematurely (3-5 weeks of age) likely due to a metabolic disorder in the liver. Instead, our data indicate that VDAC2 is important for efficient mitochondrial localisation of both BAX and BAK and is an essential promoter of BAX apoptotic function. Cells lacking VDAC2 and BAK are largely resistant to apoptosis induced by diverse stimuli including BH3-mimetics, and deletion of VDAC2 alone was sufficient to protect cells from BAX-dependent apoptosis both *in vitro* and *in vivo*. Moreover, deletion of VDAC2 accelerates MYC-driven acute myeloid leukemia confirming that VDAC2 is a key mediator of BAX apoptotic function in the context of oncogenic stress as well as chemotherapy. Thus, our data redefine the control mitochondrial apoptosis and suggest that disrupting the BAX:VDAC2 interaction is a potential mechanism of chemoresistance or to impair unwanted or damaging apoptosis for example following ischemic stroke or reperfusion injury.

M12: Relationship between cytochrome *c* and cardiolipin oxidation is highly dependent upon the experimental conditions

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Cytochrome *c* acquires a new function during apoptosis; peroxidase activity, leading to peroxidation of cardiolipin (a mitochondrial phospholipid) which is proposed to be a required step in the intrinsic apoptosis pathway[1]. The interaction of cytochrome *c* with cardiolipin in liposomes causes a conformational change in cytochrome *c*, stimulating its peroxidase activity. Three cytochrome *c* mutations have been reported, G41S, Y48H, and A51V. All have increased peroxidase activity compared to wildtype. Our results show that the cytochrome *c* variants differ in their ability to bind to cardiolipin-containing liposomes and mitoplasts, resulting in differences in cardiolipin-dependent stimulation of peroxidase activity. Moreover, using mass spectroscopy to analyse lipid oxidation, cytochrome *c*-catalysed oxidation of cardiolipin was dependent on liposome composition and on the reaction conditions. In addition, when cytochrome *c*-depleted mitoplasts were used, minimal cardiolipin oxidation was detected. Although the cytochrome *c* variants are a better peroxidase under *in vitro* conditions, the relevance of this to cardiolipin oxidation *in vivo* during apoptosis is still questionable.

1. Kagan, V. E., Tyurin, V. A., Jiang, J., Tyurina, Y. Y., Ritov, V. B., Amoscato, A. A., Osipov, A. N., Belikova, N. A., Kapralov, A. A., Kini, V., Vlasova, II, Zhao, Q., Zou, M., Di, P., Svistunenko, D. A., Kurnikov, I. V., and Borisenko, G. G. (2005) *Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors,* Nature chemical biology 1, 223-232.

M13: Lutein-mediated increased in mitochondrial respiration is associated with promotion of neuronal differentiation

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Lutein has received particular interest as it is preferentially taken up by neural tissues. Apart from its well-recognized antioxidant potential on vision, a broader role of lutein in neurogenesis has emerged recently. However, the underlying mechanism remains unclear. To explore how lutein promotes neurogenesis, the neuronal cell line SH-SY5Y was cultured with or without 10uM lutein treatment for 3 days followed by analysis of morphological, molecular and metabolic changes. We found that lutein-supplemented cells exhibited more neurites and higher expression of neuronal marker MAP2. Metabolic analysis showed that lutein increased mitochondrial basal and maximal respiration by 30% ($P<0.05$) and 40% ($P<0.05$) respectively, and proton leak by 60% ($P<0.01$), compared to control. However, ATP production remained unchanged. Moreover, real-time qPCR analysis of mitochondrial copy number showed that mitochondrial number was unchanged by lutein supplementation. Following lutein supplementation, we also found a moderate increase in reactive oxidative stress (ROS) of 12.5% ($P<0.01$) compared to the control cells. Under these conditions, mRNA levels of classical antioxidant genes remained unchanged (as measured by genome-wide microarray or targeted approach). Collectively, these results indicate lutein-mediated increase in mitochondrial respiration leads to elevated proton leak with moderately increased ROS level. Increased ROS level in the absence of a free radical scavenger response may play a positive role in the neuronal differentiation process.

M14: MLKL-dependent disruption of mitochondrial function and redox homeostasis during the initiation of TNF-mediated necroptosis

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Necroptosis is a form of regulated cell death that can be triggered by a variety of factors associated with infection and inflammation. The cellular processes leading to the necroptosis remain unclear. Early studies with tumour necrosis factor alpha (TNF), now known to be a classic necroptosis inducer, implicated mitochondria and reactive oxygen species as having a central role in cell death. We have investigated mitochondrial function and intracellular redox changes during the induction of TNF-mediated necroptosis in murine dermal fibroblasts (MDF) and murine embryonic fibroblasts (MEF). The first detectable event in these cells was phosphorylation of the mixed lineage kinase-domain like (MLKL) pseudokinase, followed closely by a burst of mitochondrial-dependent oxygen consumption, peaking at 40 and 76 min for MDF and MEF cells, respectively. Mitochondrial events were blocked by MLKL inhibitors and did not occur in fibroblasts from MLKL-knockout mice. Subsequent events included a dramatic impairment of mitochondrial respiration, oxidation of mitochondrial and cytoplasmic peroxiredoxins, indicating perturbation of redox homeostasis, and the generation of MLKL oligomers with intermolecular disulfide bonds. We propose that early MLKL phosphorylation leads to modification of mitochondrial metabolism and an intracellular oxidizing environment, and this promotes MLKL oligomerization and cell death.

M15: Mitochondrial transfer in the brain: breaking bioenergetic boundaries

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The bioenergenetic and metabolic profile of developing tumours changes dynamically to meet changing growth requirements and complex adaptive interactions with cells of the microenvironment. We have recently shown that metastatic melanoma and breast carcinoma cell models without mtDNA acquire mtDNA/intact mitochondria from adjacent normal cells[1, 2], and that this transfer is associated with respiration recovery and tumour growth. This novel discovery adds a new layer of complexity to cancer cell biology that directly impinges on adaptive remodelling following mitochondrial damage and DNA-damaging treatment regimens. To investigate intercellular mitochondrial transfer in the brain, we developed a mouse glioma model devoid of mtDNA (GL261□°) and therefore glycolytic and auxotrophic for uridine and pyruvate. When injected intracranially into syngeneic mice, tumour growth occurred between 80 and 130 days compared with 26-29 days for parental GL261 cells. Cell lines from these tumours contained mtDNA polymorphisms of syngeneic C57BL/6 mice which differ from GL261 cells at 2 mtDNA sites. These cell lines showed variable respiration recovery and tumorigenicity compared with GL261 cells when passaged intracranially. Co-culture of GL261□° with stromal cells derived from bone chips of mice transgenic for a mitochondrially-imported red fluorescent protein (mitoDsRed) showed mitochondrial uptake by GL261□° cells. Furthermore, a GFP neonatal astrocyte cell line without mtDNA (GFP NeoAstrocyte □°) acquired mitochondria from mitoDSRed bone chip-derived stromal cells, but stable lines could not be established. When injected intracranially, GFP NeoAstrocyte □° cells survived less than 7 days. We conclude that normal cells in the brain can transfer mitochondria to glioma cells without mtDNA, restoring cell respiration and tumorigenicity. Brain bioenergetics may not play by well-established cell biology rules.

1. Tan AS, et al. (2015). *Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA* Cell Metabolism 21: 81-94.
2. Dong L-F et al. (2017) *Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells*. eLife; 6: e22187.

M16: Natural models of hypoxia tolerance in New Zealand Triplefin fish

Jules Devaux, Nigel Birch, Neil Herbert, Chris Hedges, Gillian Renshaw and Tony Hickey

The adult human brain is very sensitive to hypoxia, and dies within minutes of anoxic exposure. However, aquatic animals permanently live with only 3% or less of atmospheric O_2. Moreover, intertidal environments (e.g. rockpools) periodically approach anoxia for several hours. Therefore intertidal fish resist ischemic insults that will promote brain damage and death in most other vertebrates.

Oxygen is essential for mitochondria to sustain sufficient ATP production within the vertebrate brain. With hypoxia anaerobic metabolism elevates lactate formation, as ATP and phosphocreatine stores deplete, and the brain acidifies. This triggers excitotoxicity and dysregulates Ca^{2+}, accelerating mitochondrial dehydrogenases and promoting swelling. Subsequently hypoxic stressed mitochondria can promote apoptosis or necrosis, and perhaps more importantly, anoxic mitochondria consume ATP. How intertidal fish tolerate large changes in brain pH, elevated Ca^{2+} and glutamate presents us with a natural model to explore hypoxic adaptations with a view to pathological states.

To reveal adaptations to hypoxia it is best is to compare hypoxic responses in multiple closely related species, at different biological levels of organisation. New Zealand triplefin fish (Family: Tripterygidae) provide a unique study system, having evolved by a local adaptive radiation to fill specific niches. While 25 endemic species occupy stable normoxic niches, three occupy intertidal rock pools that become hypoxic, or even anoxic at low tide. We find that at almost every level, from animal to organelle, intertidal fish are resilient to hypoxia. Here we will discuss in two parts our findings of how these charismatic little fish have adapted brain mitochondrial function to survive suffocation.

M17: Rescue of mitochondrial depletion in astrocytes by co-culture involves large scale mitochondrial transfer

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Transport of mitochondria between cells, once in the realm of science-fiction, has now been demonstrated in a variety of murine cancer models *in vivo*, including breast cancer, melanoma, leukaemia and most recently the brain tumour glioblastoma. We have looked at the ability of non-cancerous brain cells to transfer mitochondria, by generating astrocytes depleted of mitochondrial DNA (rho^0 astrocytes). The ability of different cell types to maintain survival of rho0 astrocytes under conditions of bioenergetic stress was assessed using conditioned media, two-dimension and three-dimensional co-cultures. Mitochondrial transfer was assessed using direct mitochondrial DNA staining, and by an in situ PCR. While multiple cell types were able to sustain survival and proliferation, mitochondrial transfer was not commonly observed. Instead, rapid restoration of near-complete mitochondrial networks only occurred upon co-culture with primary bone-derived cells, and appeared to be driven by cell fusion. This indicated that like their cancerous equivalents, non-transformed neural cells were also able to take up mitochondria from a heterogenous donor cell, suggesting that the phenomenon of mitochondrial transfer could be a fundamental process underlying mitochondrial and cellular biology.

M18: Single molecule genotyping for *in situ* detection and quantification of intercellular mitochondrial transfer

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The transfer of mitochondria between mammalian cells is a physiologically relevant phenomenon, however the signals driving this process are not yet clear. Cellular stress during disease or therapy may alter the rate of mitochondrial transfer between cells and surrounding tissue. Literature published in the field of intercellular mitochondrial transfer relies heavily on confocal microscopy to understand this process. Limitations of mitochondrial-specific fluorescent markers used in the field are of serious concern, thus some ambiguous data on intercellular mitochondrial transfer has been presented throughout literature.

In order to accurately detect and quantify intercellular mitochondrial transfer, a novel strategy for *in situ* molecular genotyping has been targeted toward single nucleotide polymorphisms within the mitochondrial genome (mtDNA). This strategy exploits the enhanced fidelity of an engineered, thermostable Taq ligase, that when combined with padlock oligonucleotides, serve to generate a template for target-primed rolling circle amplification of individual mtDNA molecules based on the nucleotide present at the target SNP. Our models of intercellular mitochondrial transfer have been designed to incorporate mtDNA sequence heterogeneity between 'donor' or 'recipient' cells. This enables subsequent detection of exogenous mtDNA transferred into 'recipient' cells, in bothor experiments. In a multiplexed reaction, detection of two (or more) variant mtDNAs within a model is possible and enables bidirectional analysis of intercellular mtDNA transfer. Application of this strategy allows us to trace and quantify the transfer of mitochondrial genomes, and provides robust, quantitative data to support microscopic observation of this phenomenon.

M19: Maintenance of mitochondrial function at low intracellular pH: an advantage for hypoxia tolerant species?

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Intertidal fish inhabit environments with variable O_2 supply, with acute anoxic-reoxygenation tidal exposures that mirror clinical issues. These severe challenges promote anaerobic metabolism and lactate accumulation. While lactate is an important substrate in the brain, an associated acidosis likely impacts mitochondrial function. Mitochondria (*mt*) consume oxygen (JO_2) to generate chemical (ΔpH) and electrical (Δ□m) gradients across the inner-*mt*-membrane to produce ATP, however the effects of extramitochondrial pH on brain *mt* function remains largely unexplored. We compared four species of New Zealand Triplefin fish ranging from intertidal to deeper habitats and predicted that intertidal hypoxia tolerant species (HTS) should better maintain *mt* dynamics and function with acidosis than deep-water and hypoxia sensitive species (HSS). Using fluorimetry coupled with high-resolution respirometry, we titrated lactic acid to decrease extramitochondrial pH, in parallel with buffered lactate titrations (constant pH / control). Alongside this we assessed the effect of acidosis on active pH buffering capacities, JO_2 and Δ□m of brain *mt* within permeabilised brain. As *mt* volume is rarely considered, *mt* volume estimates were incorporated into Δ□m calculations using isolated *mt*. While buffered lactate titrations had similar effects on *mt* function for all species, acidosis supressed JO_2 in HTS, which also had greater active pH buffering capacities than HSS (0.94 and 0.91 $U.mg^{-1}$ respectively). HTS also maintained Δ□m down to pH 6.2 while Δ□m of HSS fell below -110 mV from pH 6.85, most likely inhibiting ATP synthesis. Overall HTS tolerate acidosis. We further speculate that HTS may utilise extra-mitochondrial acidosis to maintain *mt* function at low pH.

M20: Mitochondrial glycerophosphate dehydrogenase facilitates pre-flight thermogenesis in *Bombus terrestris*

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Bumblebees (*Bombus terrestris*) fly at temperatures when other insects cannot. To achieve this they must warm their muscles, and while some have proposed mechanisms behind this, none fully explain the phenomenon.

Through simultaneous high resolution respirometry and fluorometry, as well as direct calorimetry, and mass spectrometry, we have described a new thermogenic mechanism in the flight muscles of *B. terrestris*. Respiratory analysis at a range of euthermic and hypothermic temperatures reveals that mitochondrial glycerophosphate dehydrogenase (mGPDH) oxidises glycerol 3-phosphate (G3P) at a substantial rate in a non-phosphorylating state. Furthermore, mGPDH has lower temperature sensitivity relative to Complex I (CI). This is evident by the 5.4-fold difference in Q_{10} between CI and mGPDH state-4 respiration. Fluorometric measurements of proton conductance revealed that inner membrane proton permeability is 3-fold higher during mGPDH-supported respiration relative to CI-supported respiration. This indicates that mGPDH function partially uncouples the electron transport system. Analysis of ATP production revealed lower P:O ratio and lower overall ATP production during G3P-supported respiration relative to CI. As oxidation of G3P yields less ATP, this energy is potentially resolved as heat. Calorimetric analysis confirms this, with 5-fold more heat released during state-4 mGPDH-supported respiration, than through state-4, CI-supported respiration. Finally, mass spectrometry of flight muscle metabolites showed that in the cold, G3P is preferentially oxidised over other mitochondrial substrates, possibly due to mGPDH's capacity to function at low temperatures and demand on the electron transport system to maintain cytosolic redox state.

Together these findings reveal a new thermogenic mechanism by which mGPDH causes mitochondrial uncoupling, high non-phosphorylating oxygen flux, and significant heat release. This resolves a long standing physiological debate around bumblebee pre-flight thermogenesis, as well as suggests a new mechanism by which tissues with high mGPDH expression may release heat. Together these findings reveal a new thermogenic mechanism by which mGPDH causes mitochondrial uncoupling, high non-phosphorylating oxygen flux and significant heat release. This answers a long standing physiological debate around bumblebee pre-flight thermogenesis, as well as suggests a new mechanism by which tissues with high mGPDH expression may release heat.

M21: Horizontal transfer of whole mitochondria restores tumorigenic potential in mtDNA-deficient cancer cells

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Recently, we showed that generation of tumours in syngeneic mice by cells devoid of mitochondrial (mt) DNA (ρ0 cells) is linked to the acquisition of the host mtDNA.[1] However, the mechanism of mtDNA movement between cells remains unresolved. To determine whether the transfer of mtDNA involves whole mitochondria, we injected B16ρ0 mouse melanoma cells into syngeneic C57BL/6N$^{su9\text{-}DsRed2}$ mice that express red fluorescent protein in their mitochondria. We document that mtDNA is acquired by transfer of whole mitochondria from the host animal, leading to normalisation of mitochondrial respiration. Additionally, knockdown of key mitochondrial complex I (NDUFV1) and complex II (SDHC) subunits by shRNA in B16ρ0 cells abolished or significantly retarded their ability to form tumours. Collectively, these results show that intact mitochondria with their mtDNA payload are transferred in the developing tumour, and provide functional evidence for an essential role of oxidative phosphorylation in cancer.[2]

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M22: Seahorse XF Technology for beginners and experts

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M23: Epigenetic mechanisms regulating exercise adaptations and mitochondrial metabolism in muscle

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A lack of physical activity is contributing to a scourge of chronic diseases including obesity, type 2 diabetes, cardiovascular disease and cancer through a loss of functional capacity and impaired muscle metabolism. Understanding the molecular responses mediating skeletal muscle adaptations to exercise presents an opportunity to identify targets to enhance functional capacity and muscle metabolism. Given that transient alterations in skeletal muscle gene expression are thought to mediate many of the beneficial adaptive effects of exercise, our efforts in understanding these responses has been focused on epigenetics and epigenetic regulators.

Our initial studies in humans showed that a signaling pathway involving histone 3 acetylation, the MEF2 transcription factor and disruption of the class IIa histone deacetylase corepressor complex is associated with exercise-induced gene expression. These findings suggested that the class IIa HDACs could be a viable target to therapeutically induce exercise adaptations. Studies in mice confirmed that genetic disruption of the class IIa HDACs corepressor complex invoked adaptive responses similar to exercise, including increased metabolic gene expression, increased oxidative capacity and lipid oxidation. Screening of compounds with similar phenotypic effects revealed Scriptaid as a compound that disrupts the class IIa HDAC corepressor complex. Chronic Scriptaid administration to mice increased exercise capacity, enhanced whole body energy expenditure and lipid oxidation and reduced blood glucose and lipids.

These studies highlight the value in understanding the molecular responses underpinning human physiology and show that pharmacological targeting of the class II HDAC corepressor complex could be a strategy to combat chronic diseases associated with physical inactivity.

M24: Placental metabolism and the role of mitochondrial glycerol 3-phosphate dehydrogenase

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During pregnancy the placenta facilitates fetal growth through transfer of nutrients and gases with the maternal circulation, and also protein and hormone synthesis. Carbohydrate oxidation and glycolysis appear to be central to healthy placental metabolism. To sustain glycolytic flux NADH must be re-oxidized to NAD, and in most tissues this occurs through linkage of the mitochondrial (*mt*) malate-aspartate shuttle with oxidative phosphorylation. There is, however, an alternative NAD regeneration system present in the placenta.

A less common means to manage the cell redox state involves the *mt* glycerol 3-phosphate dehydrogenase (mGPDH) system, which supports the glycerophosphate cycle. This cycle is generally active in tissues with requirements for fast glycolytic turnover, or substantial lipid oxidation, as it regenerates NAD faster than the malate-aspartate shuttle, and disposes of glycerol. Electrons within glycerol 3-phosphate are fed into the electron transport system (ETS) from the cytosolic side of the mitochondrial inner membrane.

The placenta has elevated mGPDH activities relative to other tissues and components of the ETS. Why the placenta should employ this cycle is not understood, but its activity could have implications for disease states of pregnancy. Using high resolution oxygraph assays we measured *mt* reactive oxygen species (ROS) production and respiration simultaneously from permeabilised placental explants. We have found that mGPDH has an additive effect on *mt* respiration on top of complexes I and II, and that mGPDH contributes the most to net ROS production, in phosphorylating states. This may result from the outward facing mGPDH. While the potential of mGPDH to produce ROS is clearly tolerated in most pregnancies, altered metabolites in disease states, such as hyperlipidemia in preeclampsia, may elevate ROS production by directing more activity through mGPDH.

M25: Assessing mitochondrial activity and response to oxidative stress in human monocyte and lymphocytes

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As we age mitochondrial function is reported to decline alongside an increase in cellular oxidative stress. Here we have used Seahorse XF Analyser technology to measure mitochondrial bioenergetics in cells obtained from freshly-drawn human blood. Density-gradient separation coupled with antibody-labelled magnetic beads was used to isolate monocytes and lymphocytes, and then mitochondrial activity was compared between cells from a young (18-25 year old) and an older (70-80 year old) cohort. Cells were also challenged with a sub-lethal dose of hydrogen peroxide to determine if there was any difference in the response of mitochondria to oxidative stress. We found that mitochondrial reserve capacity is particularly sensitive to hydrogen peroxide, with lymphocytes being significantly more vulnerable than monocytes. No age-related effect was observed between these cohorts suggesting mitochondrial metabolism in blood cells remains robust as we age.

M26: Can PBMCs predict skeletal muscle mitochondrial adaptation to training?

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Analysis of skeletal muscle mitochondrial function provides useful insight into metabolic capacity, health, and adaptation to different interventions. Direct assessment of muscle mitochondrial function typically requires a percutaneous muscle biopsy. This procedure is uncomfortable for participants, requires a trained operator, and harbours the risk of complications. As an alternative tool, we hypothesised that peripheral blood mononuclear cells (PBMCs) may also alter mitochondrial function in parallel to muscle. If so, PBMCs would provide a less-invasive estimate of muscle mitochondrial function. Moreover, whether training-induced adaptation of human skeletal muscle can be predicted from PBMCs has yet to be investigated. Therefore, we hypothesised that PBMC mitochondrial function would correlate with muscle mitochondrial function, and increase in response to a training intervention.

Resting venous blood and skeletal muscle samples were taken from ten males, before and after two weeks of high-intensity interval training (HIIT). High-resolution respirometry and fluorimetry was performed simultaneously on PBMCs and permeabilised muscle to measure rates of oxygen consumption and H_2O_2 production pre- and post-training. HIIT resulted in significant increases in state 3 and state 4 respiration in skeletal muscle, but no significant difference in PBMC respiration. To understand whether PBMC respiration could predict skeletal muscle respiration, we determined correlations between PBMC and muscle fibre respiration. Pre-training, there was no significant correlation between PBMC and skeletal muscle respiration in any state, though a trend was evident for cytochrome c oxidase ($r = 0.60$, $p = 0.06$). Post-training, complex I state 4 respiration showed a negative correlation ($r = -0.77$, $p = 0.03$), and uncoupled respiration showed a positive correlation ($r = 0.76$, $p = 0.03$). However, training-induced change in PBMC respiration did not correlate with training-induced change in skeletal muscle respiration in any state. We conclude that PBMCs mitochondrial function is a poor proxy of skeletal muscle mitochondrial function in humans.

M27: Muscling in on mitochondria and oxidative stress

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Oxidative stress, as a result of chronically high levels of reactive oxygen species (ROS) contributes to the pathogenesis of type 2 diabetes, cardiovascular disease and aging. Mitochondria are responsible for the majority of ROS production under basal (non-contraction) conditions, particularly in skeletal muscle and it appears that mitochondrial ROS is responsible for the majority of excessive ROS production in diabetes. Antioxidant supplements have been proposed as a treatment for oxidative stress and insulin resistance, but are little studied in humans. However, we now have evidence that supplementation with the antioxidant vitamin C (1 g/day) improves insulin-stimulated oxidative stress and insulin sensitivity in skeletal muscle of people with type 2 diabetes [1].

ROS are also produced during skeletal muscle contraction and have a regulatory role in the adaptations of skeletal muscle to endurance training such as increased mitochondrial biogenesis and improved antioxidant defences. Evidence from our own group [2] and others [3] supports the hypothesis that supplementation with antioxidants vitamin C (1 g/day) and vitamin E (>260IU/day) can hamper some of the cellular processes involved in increased mitochondrial biogenesis and antioxidant defences following endurance training in human skeletal muscle.

A likely explanation for this paradoxical effect of antioxidant supplements negating some of the beneficial effects of exercise training in healthy skeletal muscle but being beneficial to insulin-resistant skeletal muscle is likely due to the temporary nature of the increases in ROS with exercise and also the site(s) of ROS production during exercise which are most likely from *non-mitochondrial* sources. Understanding the paradoxical roles of ROS in the aetiology of disease and the regulation of beneficial training adaptations will provide potential targets for new pharmacological therapies into the treatment of metabolic disorders.

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