THE BIOLOGY OF AGEING

Opening Doors

Scientific workshops for young researchers

Carmona, (Seville) Spain, 11-15 March 2012

INTRODUCTION

The British Council in Spain, in collaboration with the Spanish National Research Council (CSIC) is organising a series of scientific workshops to provide opportunities for young researchers from the UK and Spain to meet face-to-face for the exchange of ideas, knowledge and information on priority topics and to explore future areas of research and collaboration.

This workshop on "The Biology of Ageing" was the tenth in the series.

PRESENTATION

The workshop programme included 9 key lectures of 30 minutes in length and 22 presentations of 20 minutes by a total of 31 researchers in the field preceded by a short welcome introduction by British Council and CSIC representatives. Networking was facilitated in advance of the meeting by the creation of a facebook group (http://www.facebook.com/?page=1&sk=messages&tid=1329128687908#!/pages/Workshop-on-the-biology-of-ageing/287833171276933?fref=ts).

The last 5 minutes of each presentation were reserved for discussion. Further opportunities for dialogue were provided at the end of each session. The workshop finished with a final discussion and possibilities for future collaboration.

The workshop was coordinated by Prof Richard Faragher, Professor of Biogerontology University of Brighton, and Chair, British Society for Research on Ageing and Dr Ignacio Torres, Director Instituto Cajal (CSIC).

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PROGRAMME

SUNDAY 11TH MARCH	
20:30:	**Informal drinks and welcome dinner at the Parador de Carmona**
MONDAY 12TH MARCH	
9.00	**Welcome** by **Rod Pryde**, British Council Director in Spain and **Miguel Ferrer Baena**, Delegate Spanish National Research Council (CSIC) in Andalusia
Session 1: (Chair, Ignacio Torres)	
9:20	**Keynote** *How does the senescence of cells cause the ageing of bodies?* **Richard Faragher** (School of Pharmacy and Biomolecular Science, University of Brighton)
9.50	**Keynote** *Mitochondria biogenesis and bioenergetics in ageing.* **Plácido Navas** (Centro Andaluz de Biología del Desarrollo, Sevilla)
10.20	*Mechanisms underlying developmental programming of ageing.* **Sarah Barnes** (University of Cambridge Metabolic Research Laboratories, Cambridge)
10.35	*Metabolic disorders and ageing: Biological phenomena that share common themes.* **James Brown** (Aston University, Birmingham)
10.50	**Keynote** *Tauopathies and dementia.* **Jesús Ávila** (Centro de Biología Molecular "Severo Ochoa", Madrid)
11.20	***Coffee and informal discussions***
Session 2: (Chair, Richard Faragher)	
11.50	**Keynote** *IGF1 and healthy brain ageing.* **Ignacio Torres** (Instituto Cajal, Madrid)
12.20	*Known unknowns: the use of functional genomics in ageing research.* **David Kipling** (Cardiff University)
12.50	*Variability in the trans-resveratrol content of red wines.* **Robert Ellis** (University Campus Suffolk)
13.05	*Altered mitochondrial dynamics and mitophagy are found in sporadic Alzheimer´s disease fibroblast.* **Patricia Martín-Maestro** (Centro de Biología Molecular "Severo Ochoa", Madrid)
13:20	***Post session discussion and networking lunch***
Session 3: (Chair, Jesús Ávila)	
15.30	**Keynote** *Ageing and the effects of steroid hormones on wound healing.* **Matthew Hardman** (University of Manchester)
16.00	*Treatment of ageing-associated neurodegenerative diseases with anti-inflammatory neuropeptides.* **Elena González- Rey** (Instituto de Parasitología y Biomedicina "López-Neyra", Armilla)
16.15	*Differential hypoxic regulation of carotid body GDNF expression with ageing: Implications for antiparkinsonian cell therapy.* **Javier Villadiego** (Instituto de Biomedicina de Sevilla-IBiS)
16.30	**Keynote** *Oestrogen and the ageing brain.* **Luis Miguel García Segura** (Instituto Cajal, Madrid)
17.00	***Coffee and post session discussions***
20.30	**Dinner at the Parador de Carmona**

TUESDAY 13TH MARCH	
Session 4: (Chair, Lynne Cox)	
9:20	**Keynote** *Human accelerated ageing disorders.* **Lynne Cox** (University of Oxford)
9.50	*Developing a model of human progeroid Werner's syndrome in the nematode worm, C. elegans.* **Hayley Lees** (Department of Biochemistry, University of Oxford, Oxford)
10.05:	*Strategies of SIRT1 enhancement against frailty in ageing and neurodegeneration.* **Rubén Corpas** (Instituto de Investigaciones Biomédicas de Barcelona)
10.20	*Olive oil polyphenols and longevity: C. elegans as a model system.* **Ana Cañuelo** (Universidad de Jaén)
10.35	*Testing the hyperfunction theory of ageing in C. elegans.* **Yila de la Guardia** (University College London)
10.50	*The role of the thioredoxin systems in Caenorhabditis elegans longevity as a paradigm of the redox regulation of ageing in metazoa.* **Antonio Miranda** (Instituto de Biomedicina de Sevilla)
11.05	*Does the honeybee provide a novel model to study effects of diet on ageing through epigenetic mechanisms?* **Luisa Wakeling** (University of Newcastle)
11.20	***Coffee and informal discussions***
Session 5: (Chair, Luis Miguel García Segura)	
12.05	*Regulation of mTOR and autophagy by lysosomal positioning.* **Viktor Korolchuk** (University of Newcastle)
12.20	*Mitochondrial translation activators modulate yeast longevity through regulation of nuclear silencing.* **Antonio Caballero** (MRC Centre for Developmental Neurobiology, King's College London)
12.35	*Impact of methionine restriction and atenolol treatment on mitochondrial oxidative stress in relation to longevity.* **Inés Sánchez- Román** (Universidad Complutense de Madrid)
12.50	*Exercise, ageing and muscular steadiness.* **Mandy Gault** (University Campus Suffolk)
13.05	**Keynote** *Ageing of the extracellular matrix.* **Michael Sherratt** (University of Manchester)
13.35	***Networking Lunch***
Session 6: (Chair, Matt Hardman)	
14.35	*Party-pitch your post-doc!* British Council led science promotion activity by **Aarathi Prasad**
16.00	**Guided tour of Carmona**
19.00	Guided tour completes, return to hotel
20.30	**Dinner at local restaurant**

WEDNESDAY 14TH MARCH	
Session 7: (Chair, Plácido Navas)	
9.20	**Keynote** *Longevity-associated genes.* **José Viña** (Universidad de Valencia)
9.50	*In vivo characterization of neuronal ubiquitin pathways: a starting point to understand ubiquitin-related neuronal diseases.* **Ugo Mayor** (CIC bioGUNE, Bilbao)
10.05	*Osteoporosis: Evaluation of genetic polymorphisms.* **Miriam Martínez** (Hospital Universitario Virgen de la Arrixaca, Murcia)
10.20	*DNA polymerase mu, a member of the NHEJ pathway, plays a relevant role in murine ageing.* **Antonio Bernad** (Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid)
10.35	*Identification and characterisation of new models for presbycusis.* **Prashanthini Shanthakumar** (Medical Research Council Harwell)
10.50	*Antioxidants and cell function.* **Patricia Santofimia** (Facultad de Veterinaria de Cáceres, Universidad de Extremadura, Cáceres)
11.05	**Coffee and networking**
Session 8: (Chair, David Kipling) FUNDING OPPORTUNTIES	
11.30	*Funding opportunities and advice from the UK*, **Richard Faragher** (University of Brighton)
12.15	*Funding opportunities and advice from Spain*, **Julio Barbas** (Ministerio de Economía y Competitividad)
13.00	*Group discussion: emergent opportunities.*
13.30	***Networking Lunch***
Session 9: (Chair, Richard Faragher) NEXT STEPS	
15.30	*Needs and wants for further collaboration* (all-facilitated by organisers and British Council)
16.30	***Coffee***
17.00	***General summary and conclusions***
20.30	***Dinner at the Parador de Carmona***

SUMMARY OF DISCUSSIONS

Discussions within the meeting covered three distinct areas. The first of these was the potential for effective collaboration on the purely scientific areas of participants interests. The second was the discussion of the enabling measures which would be most effective in delivering these. The last was the potential for effective dissemination of scientific findings to a broader audience.

The participation and active engagement of British Council representatives was important in facilitating all of these activities. The scientific programme was opened by the director of the British Council Mr Roderick Pryde (giving the meeting an appropriate sense of gravity) and the scientific dissemination activities for early career researchers were facilitated by Dr Aarathi Prasad (Advisor, life sciences and natural resources).

The scientific discussions recognised the importance of the insulin IGF-I signalling pathway in driving enhanced longevity in a variety of models. It was recognised that the attendees had a broad range of both techniques and model systems on which to draw. Given the scale of the challenge presented by the ageing of the population in Spain, the United Kingdom, and the European Union it was felt that modest investment in a collaborative approach would produce considerable benefits for both the Spanish and British research communities. The collaborations outlined in this report are the direct result of these discussions.

At the same time, it was recognised that the workshop was taking place at a point in which the funding structures and organisation of the National science base in Spain were undergoing significant restructuring as a consequence of the overall economic situation. The presentation by Julio Barbas (Ministry of Economy) on these changes together with that by Richard Faragher on the UK science base made it clear that effective facilitation measures designed to take collaboration forward would need to be based on:

- The effective advance dissemination of calls for proposals which could allow the participants to submit jointly (e.g. in the run-up to Horizon 2020 and the use of enabling measures designed to underpin the creation of a single European research area).
- The facilitation by funders of additional workshops allowing highly focused topics to be worked up into credible transnational proposals (e.g. through the use of the COST activity).
- The maximisation of routes for productive engagement between the scientific communities lying outside the routine activities of the funding bodies (e.g. through more active engagement with the UK FCO).

ABSTRACTS

Richard Faragher, Replicative senescence and its links to mammalian ageing

Plácido Navas, Mitochondria biogenesis and bioenergetics in ageing

Sarah Barnes, Mechanisms underlying developmental programming of ageing

James Brown, Metabolic disorders and ageing: Biological phenomena that share common themes

Jesús Ávila, Tauopathies and dementia

Ignacio Torres, IGF-I and healthy brain aging

David Kipling, The application of bioinformatics to ageing and disease

Robert Ellis, Variability in the trans-resveratrol content of red wines

Patricia Martín-Maestro, Altered mitochondrial dynamics and mitophagy are found in sporadic Alzheimer´s disease fibroblast

Matthew Hardman, Ageing and the effects of steroid hormones on wound healing

Elena González- Rey, Treatment of ageing-associated neurodegenerative diseases with anti-inflammatory neuropeptides

Javier Villadiego, Differential hypoxic regulation of carotid body GDNF expression with ageing: Implications for antiparkinsonian cell therapy

Luis Miguel García Segura, Oestrogen and the ageing brain

Lynne Cox, Human accelerated ageing disorders

Hayley Lees, Developing a model of human progeroid Werner's syndrome in the nematode worm, C. elegans

Rubén Corpas, Strategies of SIRT1 enhancement against frailty in ageing and neurodegeneration

Ana Cañuelo, Olive oil polyphenols and longevity: C. elegans as a model system

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Antonio Miranda, The role of the thioredoxin systems in Caenorhabditis elegans longevity as a paradigm of the redox regulation of ageing in metazoa

Luisa Wakeling, Does the honeybee provide a novel model to study effects of diet on ageing through epigenetic mechanisms?

Viktor Korolchuk, Regulation of mTOR and autophagy by lysosomal positioning

Antonio Caballero, Mitochondrial translation activators modulate yeast longevity through regulation of nuclear silencing

Inés Sánchez- Román, Impact of Methionine Restriction and Atenolol treatment on mitochondrial oxidative stress in relation to longevity

Mandy Gault, Eccentric Exercise, Ageing and Muscular Steadiness

Michael Sherratt, Age-related stiffening of the extracellular matrix

José Viña, Longevity-associated genes

Ugo Mayor, In vivo Characterization of Neuronal Ubiquitin Pathways: a starting point to understand ubiquitin-related neuronal diseases

Miriam Martínez, Osteoporosis: Evaluation of genetic polymorphisms

Antonio Bernad, DNA polymerase mu, a member of the NHEJ pathway, plays a relevant role in murine aging

Prashanthini Shanthakumar, Identification and characterisation of new models for presbycusis

Patricia Santofimia, Antioxidants and cell function

Replicative senescence and its links to mammalian ageing

Richard Faragher
University of Brighton

Any biological process that is postulated to play a causal role in ageing must satisfy three fundamental mechanistic criteria. Firstly, the process in question must take place in vivo, secondly it must be capable of exerting degenerative effects and lastly altering the rate at which organismal ageing occurs should also alter the rate at which the candidate mechanism operates (and vice versa). The progressive accumulation of senescent cells has long been proposed to act as an ageing mechanism. Such cells display a radically altered transcriptome compared to their growing counterparts and as a result display a profoundly altered phenotype which could plausibly exert degenerative effects. Whilst tremendous progress has been made in recent years in understanding the molecular mechanisms that regulate entry into the senescent state attempts to determine whether senescent cells can act as causal agents of mammalian ageing have been much more limited. However, those data which do are exist are coherent and demonstrate:

- That replicative senescence exists in vivo.
- That significant numbers of cells become senescent during the lifespan of mammals.
- That the altered phenotype of senescent cells can trigger degenerative pathology in vivo.

Perhaps the best evidence for a causal relationship between replicative senescence and organsimal ageing is still provided by Werner's syndrome, a rare autosomal recessive disorder that is characterised by the premature development of multiple age-related pathologies and premature fibroblast senescence. Together with our collaborators we have demonstrated that the accumulation of senescent cells can plausibly explain some, but not all of the ageing phenotypes seen in this disorder.

Mitochondria biogenesis and bioenergetics in ageing

Plácido Navas
Centro Andaluz de Biología del Desarrollo

People show evident cosmetic and functional decline as age, and an important part of this phenomenon is due to the loss of energy supply to maintain body structure and functions. Most of useful energy production is in mitochondria and its homeostasis must be considered when we study aging. Mitochondria homeostasis is an integrated process that involves both biogenesis and mitophagy, but also fusion and fission events. Mitochondria could be considered as the operative system of the cell that coordinates the main metabolic pathways. To study these pathways and the molecular mechanisms associated to age, different approaches were used including caloric restriction and resveratrol treatments. Calorie restriction is a non-genetic intervention that extends longevity in most of the animals and also increase health by attenuating age associated diseases including cancer. Calorie restriction induces the biogenesis of efficient mitochondria and modulates pyridine nucleotides homeostasis by the coordination of respiratory chain and plasma membrane electron transport activities. This intervention also activates sirtuins as a key factor probably by the supply of NAD+ throughout these activities. Resveratrol mimics calorie restriction and prevents obesity-associated dysfunctions. Resveratrol induces mitochondria biogenesis in mice, shifts the metabolism of cancer cells to respiration, and activates plasma membrane electron transport. As a consequence also contributes to increase NAD+ as the key component of activation of sirtuins to coordinate cellular bioenergetics and genome regulation.

Mechanisms underlying developmental programming of ageing

Sarah Barnes
University of Cambridge, Metabolic Research Laboratories

I am currently investigated novel and known gene expression patterns in relation to age in the liver of mice. Of these genes a subset has been shown to change in relation to maternal

diet. Three maternal diet groups are studied, a normal 20% protein diet is consumed by the mother during pregnancy and lactation, a reduced 8% protein diet is consumed in pregnancy and then the pups are cross fostered to a 20% protein fed mother during lactation (recuperated) and in the last groups a normal 20% protein diet is consumed in pregnancy, but the pups are lactated by a low protein fed mother (Postnatal low protein (PLP)). All diets are isocaloric. The most interesting phenotype in these animals is in longevity. The PLP animals live significantly longer compared to control, conversely the recuperated live shorter than control. Several interesting genes have been observed to change between diets during the ageing process. One of the most interesting is cell death-inducing DFFA-like effector a *(Cidea)*, which is up regulated in old age. In this liver, a much higher up regulation occurs in recuperated mice than control. This gene plays a role in lipid homeostasis and a better lipid profile in the liver is observed in knockout studies. *Cidea* appears to be controlled by epigenetics, but further investigation is looking to see if it is a direct affect.

If possible I would like to look at the liver of old aged methionine restricted rats and controls to see if *Cidea* expression is conserved between rodents and if it is, does the reduction in methionine reduce the expression of Cidea (Ines Sanchez-Roman – Universidad Complutense de Madrid)

Metabolic disorders and ageing: Biological phenomena that share common themes

James Brown
Aston Research Centre for Healthy Ageing, Aston University

Although many estimates suggest that both the UK and Spain will see large increases in the numbers of older adults in the next few decades, the concomitant increase in obesity levels may prevent this from happening. Currently, in both the UK and Spain, around 1 in 4 people are obese. Interestingly, many of the pathways and signalling molecules associated with metabolic disorders such as diabetes and obesity are also implicated in ageing (including insulin/IGF-I, mTor, PTEN and oestrogen). Additionally, several previous studies have shown that metabolic parameters such as obesity, circulating glucose levels, body mass index and insulin correlate with telomere length in human subjects. These findings suggest that energy balance is key to both normal metabolism and the ageing process.

Tauopathies and dementia

Jesús Ávila
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Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED)

Tau protein is a brain microtubule associated protein. Upon its binding tau stabilizes the microtubules, a component of the cytoskeleton. Tau facilitates, together with other microtubule associated proteins (MAPs), the formation and the presence of the cytoplasmic extensions known as axon and dendrites in a neuron. To look for its specific functions in vivo, a mouse lacking tau was characterized and in the analysis of its adult neurogenesis a delay in differentiation and migration of newborn neurons was found in that tau k.o. It suggests that tau play a role in adult neurogenesis. On the other hand, overexpression of tau, in regions like the dentate gyrus, could have negative consequences. In a transgenic mouse, the overexpression of human tau, results in the degeneration of the ventral dentate gyrus. The consequences of that degeneration were changes in the mood and behavior of that transgenic mouse. Also, the presence of an overexcess of tau modified by phosphorylation was analyzed in dentate gyrus. In this case, phospho tau promotes the degeneration of the dorsal region of the dentate gyrus. The consequences of this degeneration were a cognitive impairment and a memory loss in this genetically modified mouse. This mouse has been used as a model to study some features of tau associated pathology found in Alzheimer disease. On the other hand in Alzheimer disease there is a tau pathology spreading from the hippocampal to neocortical areas. A model for the progression of that pathology was postulated. It suggests that extracellular tau itself could be the agent that propagates tau pathology in disorders like Alzheimer disease on other tauopathies.

IGF-I and healthy brain ageing

Ignacio Torres
Instituto Cajal (CSIC), Madrid

Insulin-like growth factor I (IGF-I) belongs to the ancient insulin family of peptides already present in nematodes (worms) and actively involved in aging processes through their key regulatory role in energy allocation. In the adult brain, IGF-I exerts a wide variety of actions as a central modulator of neural homeostasis. Specifically, IGF-I is involved in tissue remodeling, energy availability and higher brain functions such as learning and memory. IGF-I levels gradually decline along aging in all mammals. This protracted decay may be related to brain aging as all the characteristic traits associated to old age can be satisfactorily explained by declining IGF-I input to the brain. These include loss of cognitive function, tissue atrophy and impaired proteostasis as IGF-I modulates synaptic plasticity, neurogenesis and angiogenesis, and protein homeostasis, respectively. Our knowledge of physiological factors controlling IGF-I input to the brain, such as exercise, balanced diets, or mental activity, allow us to design life-style strategies for healthy brain aging based on stimulation of IGF-I actions along the aging process.

Known unknowns: the use of functional genomics in ageing research

David Kipling
School of Medicine, Cardiff University

Scientific research often involves the testing of hypotheses. Because this requires some degree of prior knowledge in order to formulate a plausible hypothesis, totally unexpected findings are rare. Another approach can be termed “hypothesis generation”, which has a greater potential to discover the unexpected, but this can be problematic for funders because of the perceptions of risk, despite experience having shown that such unexpected findings do exist - they are “known unknowns”.

One set of tools commonly used in hypothesis-generation approaches are those of functional genomics. These include whole-genome analyses at the epigenome, proteome and transcriptome levels, and of DNA sequence variation (typically with a focus on sequence changes that affect biological function). Here we present three examples to illustrate the use of whole-genome functional genomics approaches to investigate the biology of ageing in the human cornea. In the first, we used comparative transcriptome analysis to produce a 54-gene transcriptional fingerprint that distinguishes corneal keratocytes from corneal endothelium, and from other fibroblastoid cells in the body. Using this signature we showed that, although widespread changes in gene expression occur at replicative senescence in keratocytes, there are few changes in the expression of these 54 genes, which are closely associated with keratocyte-specific function. This is interesting from the perspective of understanding how replicative senescence may alter differentiated cell functions. In the second example we undertook a transcriptome analysis of senescence in corneal endothelial cells. Over-Representation Analysis (ORA) revealed an enrichment of p53-dependent transcripts in the senescence-specific geneset. This is consistent with interventional data using HPV E6 that demonstrated a role for p53 in triggering senescence in this cell type.

To understand senescence and thus how it may have consequence in ageing, it is vital to understand the selective forces that caused its evolution, and in particular the role of senescence as a tumour suppressor mechanism. In the third example we over-expressed CDK4 in human corneal endothelial cells. This extended cellular lifespan until a second, downstream proliferative lifespan barrier (PLB) was reached. Cells at this downstream PLB show a familiar senescence-associated secretory phenotype (SASP, notably involving increase production and secretion of IL-1 and IL-6). However, transcriptome analysis also revealed an unexpected up-regulation of interferon-stimulated genes at this downstream PLB. Many of the genes up-regulated are involved in antigen processing, transport and presentation (via MHC Class 1).

We speculate that the gene expression changes at this downstream PLB act to promote immunesurveillance and clearance of senescent cells carrying potentially oncogenic lesions in two ways. First, a general inflammatory response (canonical SASP) that acts to recruit immune effector cells to the vicinity. Second, an enhancement of antigen presentation so

as to highlight potential neoplastic cells to the adaptive immune system for clearance. The challenge for ageing research is to take the conceptual framework of antagonistic pleiotropy (the concept that ageing is a non-selected side-effect of enhanced early-life reproductive success) and to understand how the complex changes that occur at senescence that evolved to provide a tumour suppressor mechanism can also contribute to the ageing of tissues.

Vine age affects trans-resveratrol concentration in red wine

Robert Ellis
University Campus Suffolk

Studies have identified trans-resveratrol (3,4',5 trihydroxystilbene), a polyphenolic molecule found in red wine, to have potentially beneficial effects on human health. This has led to suggestions that red wine could be considered a plausible functional food. Levels of trans-resveratrol in grapes are affected by; variety, 'terroir' factors such as soil type, altitude, and climate. It has also been demonstrated that exposure to fungal disease such as Botrytis *cinerea* where trans-resveratrol is produced as a phytoalexin, can affect trans-resveratrol production. Few studies make comparisons between *trans-resveratrol* concentrations of wines produced from the grapes of very old vines to wine made from the fruit of younger vines. This study sought to compare trans-resveratrol in Tempranillo varietal wines from old and new vines grown and processed under similar conditions. The wine produced from old vines was from vines ranging between 48 and 80 years old. The wine produced from young vines was from those only 9 years old. HPLC analysis of *trans*-resveratrol concentrations were by direct injection of filtered, diluted wines. Results indicated that the mean trans-resveratrol were significantly different ($p<0.05$), with mean concentrations of the wine from the old vines being 0.74mg/l compared to 0.42mg/l in the wine produced from younger vines. Higher levels of trans-resveratrol in older vines may be due to greater lifelong exposure to *Botrytis cinerea* and subsequent elevated trans-resveratrol synthesis. Further research is required to fully elucidate the contribution of vine age and disease exposure to mechanisms governing trans-resveratrol synthesis. This is of particular importance as smaller vineyards are encouraged to grub up old vines that tend to produce fewer fruits in favour of new, high yielding vines with a potential loss of quality.

Altered mitochondrial dynamics and mitophagy are found in sporadic Alzheimer´s disease fibroblast

Patricia Martín-Maestro
Centro de Biología Molecular Severo Ochoa (CSIC-UAM)

Alzheimer's disease is a progressive neurodegenerative disorder and the leading cause of dementia. There have been many hypotheses proposed to explain the origins of AD. One of the most debated hypotheses implicates mitochondrial dysfunctions and oxidative stress as early events in AD development. To carry out this work we used 14 lines of human skin fibroblasts obtained from Coriell institute. The alzheimer´s disease samples contained both sporadic and mutation in presenilin 1 with their correspondent healthy age-matched samples. Because there is an increased oxidative stress in fibroblasts from AD through oxyblot analysis we observed that AD cells showed higher oxidized proteins in normal cell conditions. Then, we studied a possible defect in mitochondrial dynamic. Analysis revealed a delayed recovery of mitochondrial filamentous morphology and less membrane potential recovery in AD fibroblasts. Moreover, we analyzed autophagic flow because defective lysosomal proteolysis represents a basis for pathogenic protein accumulations in AD. Results showed an accumulation of autophagic vacuoles shown in AD and a diminished autophagy flow when the lysosomal degradation was inhibited. Additionally, we did not observe an impairment in lysosomal pH in the case of sAD. Due to the relevance of autophagy and mitochondrial dysfunction, we studied a molecular marker involved in mitophagy. Parkin levels have shown to be decreased in AD fibroblasts with respect to NHFs. Our previous results indicate that a defect in mitophagy may take place in AD fibroblasts. This work demonstrates that some of the defects described for AD neurons can be found in fibroblast and we will study if they can also be observed in the induced neurons.

Ageing and the effects of steroid hormones on wound healing

Matthew Hardman, Mat Hardman
Faculty of Life Sciences,
University of Manchester

With increasing age our skin undergoes structural and functional changes that result in decreased regenerative capacity. In a significant proportion of the elderly population healing fails to progress, leading to chronic wounds (eg. venous or diabetic ulcers). Pathological healing in the elderly represents a major area of unmet clinical need that results in substantial morbidity and mortality. Human steroid hormone profiles also undergo pronounced changes with advancing age, particularly following menopause in women. Using a combination of in vitro, and in vivo models we have explored the role of estrogen deficiency, skin ageing and estrogen-receptor-mediated signalling in delayed healing. Our recent data show that estrogen deficiency leads to pronounced acceleration of skin ageing, which in turn delays repair. Pharmacological and transgenic manipulation of estrogen signalling has revealed divergent roles for the two estrogen receptors in skin homeostasis and repair. An improved understanding of the complexities of estrogen signalling during skin ageing and repair has a) revealed important novel mechanistic aspects of chronic wound development and b) allowed us to develop and test new therapeutic strategies to promote skin healing.

Treatment of ageing-associated neurodegenerative diseases with anti-inflammatory neuropeptides

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Cortistatin (CST) and Adrenomedullin (AM) are neuropeptides synthesized by neural and immune cells with common characteristics to be considered as potential regulators of immune responses. They are potent anti-inflammatory agents, which modulate autorreactive responses and induce immune tolerance. In this sense, both neuropeptides have been successfully used as therapeutic agents in animal models of inflammatory and autoimmune diseases such as sepsis, rheumathoid arthritis or inflammatory bowel disease. In agreement, alterations in the levels of these neuropeptides and/or their receptors lead to changes in susceptibility to suffer inflammatory and autoimmune diseases, suggesting a crucial role for AM and CST in health and disease. One crucial factor affecting the levels of AM and CST is ageing. Ageing is a physiological process that, depending on environmental, genetic and other factors, could be associated with chronic neuroinflammation. Ageing is the main risk factor leading to the development and progression of some age-related neurodegenerative disorders such as Alzheimer´s or Parkinson´s disease (AD and PD, respectively). In these diseases, dysregulated microglia (the main mediator of immune responses in the brain) with altered phenotype loses its immune and neuroprotective functions leading to chronic inflammatory response, neurotoxicity and subsequent cognitive impairment or altered motor coordination. Previous reports described the presence of AM and CST in cortex, hippocampus and hypothalamus, main brain areas related with learning, memory and emotional behaviour, which are critically affected on AD and PD. In fact, CST levels are reduced in post-mortem samples of temporal lobe of AD patients. Both neuropeptides showed cytoprotective effects after neuronal cell damage and also induced neurotrophic factors, suggesting an important role in neurodegenerative diseases. In addition, lack of AM influenced growth and differentiation of adult neural stem/progenitor cells, mainly affecting the oligodendrocytes population. Based on these results, our hypothesis is that AM and CST have a critical role in neurodegenerative diseases and that alterations in their levels can influence neuroinflammatory processes underlying AD and PD. In our laboratory, we have recently demonstrated a therapeutic effect of AM and CST when used in the animal model for multiple sclerosis (experimental autoimmune encephalomyelitis, EAE), an example of neuroinflammatory and neurodegenerative disease. AM and CST treatment diminished the infiltration of inflammatory cells in the brain and spinal cord parenchyma, decreasing expression of proinflammatory mediators and the subsequent demyelinization characteristic of EAE. AM and CST also downregulate the autoimmune component of the disease. *In vitro*

experiments showed that both neuropeptides can be produced by microglia and astrocytes and are regulated under inflammatory conditions. In addition, AM and CST can downregulate activation of microglia and astrocytes stimulated with inflammatory stimuli. Both neuropeptides protect these cells from oxidative stress. They also protect oligodendrocytes from cell death when inflammation is present. These results show that in addition to their immunomodulatory role, the therapeutic effect of AM and CST is also related with their de-activating role on resident stimulated glial populations during MS, favouring oligodendrocytes survival and its neuroregenerative function. These effects suggest a potential role of AM and CST as therapeutic agents to be used in the treatment for other neuroinflammatory disorders such as AD and PD, in which there is an important neurodegenerative but also neuroinflammatory component, and in which a neuroregenerative process is highly desirable.

Differential hypoxic regulation of carotid body GDNF expression with ageing: Implications for antiparkinsonian cell therapy

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The glial cell line-derived neurotrophic factor (GDNF) induces a notable protective effect on dopaminergic neurons in rodent and primate models of Parkinson's disease (PD). Similarly, carotid body (CB) transplants produce trophic protection and restoration of dopaminergic neurons in parkinsonian animals. Compatible with that are the high GDNF levels encountered in the CB implants. Moreover, CB cells are physiologically resistant to hypoxia, a normal environmental condition in the brain that is accentuated inside intracerebral grafts. Here, we report that chronic environmental hypoxia (PO2 ~75 mmHg) induced an up-regulation of CB GDNF expression in young mice (2-3 months old). Surprisingly, the same treatment resulted in decreased CB GDNF expression in aged mice (>14 months old). This differential regulation of GDNF expression with ageing was also observed in intrastriatal CB grafts and affects the efficacy of antiparkinsonian CB cell therapy. We have found that young CB implants induced an important protection of the nigrostriatal dopaminergic neurons of MPTP treated mice while old CB grafts failed to produce a significant effect. In addition, we have demonstrated that young CB implants are able to induce trophic protection of dopaminergic nigrostriatal neurons of aged host parkinsonian mice. Finally, we studied the role of hypoxia-related transcription factors in the regulation of GDNF expression. These findings are in accord with previous clinical trials, where the efficacy of CB autotrasplantation in PD patients was inversely related to patient age. Thus, transplantation of dopaminergic CB glomus cells appears as an excellent method to produce intracerebral delivery of GDNF, but the age of implanted CB cells should be taken into consideration for this purpose.

Oestrogen and the ageing brain

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Results from animal experiments showing that estradiol is neuroprotective, were challenged 10 years ago by findings of the Women's Health Initiative study, indicating an increased risk of dementia and stroke in women over 65 years of age taking conjugated equine estrogens. Our understanding of the complex signaling of estradiol in neural cells has recently clarified the causes of this discrepancy. New data indicate that estradiol may lose its neuroprotective activity, or even increase neural damage, a situation that depends on age-associated modifications in the levels or the signaling of other molecules that modulate estradiol action, such as insulin-like growth factor-I (IGF-I). In addition, neuronal damage is associated with changes in the expression of estrogen receptors and IGF-I receptors and with changes in the local synthesis of estradiol and IGF-I in the nervous system. These studies highlight the complex neuroprotective mechanisms of estradiol and suggest a window of opportunity during which effective hormonal therapy will promote brain function and cognition.

Human accelerated ageing disorders

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Human ageing is challenging to study at a molecular and cellular level because of the variable contribution of a wide range of genes to ageing. Human accelerated ageing disorders, particularly the segmental progerias Hutchinson-Gilford progeria, Bloom syndrome, Rothmund-Thompson syndrome and Werner syndrome, overcome this genetic limitation. Each is caused by mutation of a single gene, which results in recapitulation of many features of normal ageing. In particular, Werner syndrome (WS) is an adult onset progeria which most closely resembles normal ageing, with patients developing many of the signs and diseases of old age in early adulthood. The protein mutated in Werner syndrome is WRN, which possesses both helicase and exonuclease activities.

By analysing individual DNA molecules during replication, we have shown that WRN is required for efficient DNA replication, probably acting to prevent replication fork stalling or to promote fork restart. We have used ectopic expression of a bacterial Holliday junction nuclease to overcome both replicative and proliferative defects in WS cells, demonstrating that WS defects can be overcome at the molecular level by replacing nuclease activity. However, while WS presents a useful model of ageing, it is impossible to move beyond cell studies to experiment at the whole organism level in humans, hence there is a need to develop new systems in which we can study the role(s) of WRN and why their loss causes premature ageing. We have identified a gene in the fruit fly *Drosophila melanogaster* that is the orthologue of human WRN exonuclease, and verified biochemically that the encoded fly protein acts in a very similar way to human WRN exonuclease, degrading the same DNA substrates and using equivalent amino acids for catalysis. We have extended these in vitro studies to test the effect of WRN exonuclease loss or mutation on flies, showing an increase in recombination consistent with the hyper-recombinant phenotype and elevated cancer incidence seen in human WS. In addition, we have identified a nuclear division defect in flies lacking WRN exo. Thus by combining biochemical and whole organism studies, we can directly relate the activity of WRN *in vitro* to its role in vivo throughout the lifecourse of a metazoan animal.

Strategies of SIRT1 enhancement against frailty in ageing and neurodegeneration

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In aging and in the diseases associated with the elderly there is a progressive cognitive loss caused by deteriorating brain function. At present, the progressive gain in life expectancy has led to an increase of the incidence of aging-related neurodegenerative diseases. Recent research into the science of aging has identified genes and pathways that appear to control the aging and neurodegenerative process. One pathway that has recently drawn much attention is mediated by the protein Sirtuin1 (SIRT1), a member of the sirtuin family of protein deacetylases involved in lifespan extension and also considered a neuroprotective protein. The senescence-accelerated prone 8 (SAMP8) mice, an accelerated aging model, present an early age-related pattern and shows overproduction of Aβ and p-tau. In this study, we performed functional assays of mitochondrial activity and oxidative stress in neuron cultures from SAMP8 and senescence-accelerated-resistant (SAMR1) mice models to examine the status of neurons with emphasis on the mitochondrial function. The SAMP8 mitochondria presented lower membrane potential and higher vulnerability to mitochondrial damaging agents than SAMR1 mitochondria. This increased vulnerability indicated the presence of a senescence-associated frailty stage in SAMP8 neurons. We assayed the neuroprotective effects of resveratrol, a known Sirtuin 1 enhancer agent, and those of melatonin, also effective in anti-aging mechanisms. Here we show

that both agents, resveratrol and melatonin, protected against the senescence-associated mitochondrial frailty stage in SAMP8 mouse neurons, and that the mechanisms involved includes the enhancement of Sirtuin1 expression and antioxidant mechanisms. New studies of Sirtuin1 overexpression by using lentiviral vectors in hippocampal neurons of 3xTg-AD mice (the mouse model of Alzheimer's disease) are ongoing. The beneficial effects of SIRT1 enhancement make this molecule a promising target for research against frailty in aging and neurodegeneration.

Olive oil and Longevity: C. elegans as a model system

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Plant polyphenols are known to induce protection in a wide range of pathologies including cancer, cardiovascular disease and neurodegenerative disorders. Recently, several phenolic compounds have been shown to also increase life expectancy and stress resistance in simple model organisms. Although not long ago these effects were believed to be solely due to the antioxidant and anti-inflammatory properties present in most of these compounds, growing evidence points towards an antioxidant-independent action, most likely involving changes in gene expression patterns and affecting different signal transduction cascades.

Tyrosol and hydroxytyrosol, in simple forms or in conjugates, are the main phenolic compounds present in Extra Virgin Olive Oil (EVOO), with reported protective effects in human health such as inhibition of LDL oxidation and platelet aggregation, among others. Nevertheless, the potential effects of these compounds on longevity in a whole organism had not been studied before. In order to investigate the effects of tyrosol and hydroxytyrosol on longevity, we decided to use the nematode Caenorhabditis elegans, a well characterized model organism which facilitates lifespan assays and molecular analyses. Our results demonstrate that one of the specific tyrosol concentrations assayed was able to induce a significant increase in the median lifespan of C. elegans. This phenol also delayed the onset of a typical marker of aging in these nematodes and increased survival to heat and oxidative stress. We also found that tyrosol induces a significant up-regulation of a small Heat Shock Protein (sHSPs) family gene, whose expression is highly controlled by the Insulin/Igf-1 (IIS) signaling pathway, known to modulate longevity in this and other organisms. In addition, we have started to perform lifespan assays with mutant strains which will provide useful information regarding the specific genetic requirements or molecular pathways involved in tyrosol effects in this model organism.

In conclusion, this study demonstrates for the first time that a single phenolic compound from EVOO is able to promote longevity in an animal model. Our results suggest that this effect may be related to the ability of tyrosol to induce the expression of specific genes directly involved in key longevity regulation pathways.

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Testing the hyperfunction theory of ageing in C. elegans

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A highly regarded theory in the ageing field proposes that the accumulation of molecular damage caused by reactive oxygen species (ROS) leads to ageing. This theory also predicts that processes that aid in detoxification such as antioxidant treatments and enzymes should influence lifespan. However, experimental tests using the model organism *Caenorhabitis elegans* looking at the role of antioxidants have obtained mixed results, some of which contradict the theory. An alternative theory proposed by Mikhail Blagosklonny suggests that ageing is caused by quasi-programmed continuation of growth promoting pathways such as insulin signalling (IIS) and target of rapamycin (TOR) that lead to an increase in biosynthesis, causing age-related pathologies.

This needless continuation of tightly regulated developmental programs has been termed 'hyperfunction' and lead to hypertrophy or overgrowth. Previously reported age-related changes in *C. elegans* could be attributed to 'hyperfunction' such as accumulation of yolk protein after the cessation of oocyte production and the progressive disintegration of the gonad in hermaphrodite worms. In order to increase oocyte quality, apoptosis removes germ cells to provide cytoplasm for developing oocytes. Failure to-switch off this mechanism causes quasi-programmed apoptosis, which is responsible for disintegration of the gonad in hermaphrodite worms. Blocking programmed cell death using apoptosis defective mutant *ced-3(n717)* protects from gonad disintegration.

The role of the thioredoxin systems in Caenorhabditis elegans longevity as a paradigm of the redox regulation of ageing in metazoa

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Thioredoxins comprise a conserved family of redox regulators involved in many biological processes including stress resistance and aging. In this workshop, we have reported our recent data on the role of several members of the thioredoxin system in *Caenorhabditis elegans* longevity and in worm models of aging-associated neurodegenerative diseases such as Alzheimer´s, Parkinson´s and Huntington´s Diseases. Thioredoxin-1 *(trx-1)* is exclusively expressed in ASJ sensory neurons and it mediates dauer formation impacting in the three major dauer pathways: cGMP, TGF-beta and insulin pathways. Consistent with a role in the formation of the long-lived dauer larvae, *trx-1* has been found to modulate *C. elegans* longevity. Thus, worms carrying the null allele *trx-1 (ok1449)* are short lived while overexpression of TRX-1 causes a mild increase in mean and maximum lifespan. Interestingly, *trx-1 (ok1449)* mutants fully suppress the extended lifespan of *eat-2 (ad1116)* mutants, a genetic surrogate of calorie restriction and also suppress by approximately 70% the extended lifespan of the insulin receptor daf-2 (e1370) mutants. We are currently trying to identify by which mechanism *trx-1* impacts lifespan in these two apparently unrelated paradigms of extended longevity.

On the other hand, we also reported on the characterization of the *C. elegans* mitochondrial thioredoxin system. Interestingly, we have found that mitochondrial thioredoxin reductase *trxr-2* plays a protective role in a worm model of Alzheimer´s Disease (AD) as trxr-2 downregulation by RNAi and *trxr-2* null mutants increase the aging-dependent paralysis phenotype of the AD worms. Interestingly, TRXR-2 overexpression does not improve paralysis but causes a dramatic decrease of both total Abeta and beta-amyloid deposits. This result might be relevant to the human scenario of the most prevalent neurodegenerative disease worldwide.

Finally, we have shown that dnj-27, the *C. elegans* orthologue of the ER-resident protein ERdj5, is a major modulator of protein aggregation in cytoplasm. *dnj-27* RNAi downregulation causes a significant increase of Abeta, alpha-synuclein::YFP and polyQ::YFP cytoplasmic aggregation. This increased protein aggregation correlates with enhanced motility impairment. Conversely, overexpression of DNJ-27 alleviates both the aggregation and motility phenotypes. As a whole, our results suggest that redox regulation is a major mechanism that impacts aging and associated ageing-related diseases and places the *C. elegans* model as an ideal organism to study the function of the thioredoxin system in these pathologies.

Does the honeybee provide a novel model to study effects of diet on ageing through epigenetic mechanisms?

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Effects of diet mediated through an epigenetic process are profound in the honeybee and the reason for the development from genetically-identical larvae of the large, reproductive and long-lived queen versus the small, sterile and transient worker. During larval development, queens have unlimited access to royal jelly whereas worker larvae are fed a more restricted mixture of gland secretions and crop contents. Components of royal jelly include the

protein royal actin, which was able to induce differentiation of larvae into queens. The fact that the fundamental process underlying the response to royal jelly is DNA methylation was demonstrated in ground-breaking research in which reduced expression of the DNA-methylating enzyme Dnmt3, achieved through injection of larvae with siRNA, changed profoundly the developmental trajectory resulting in the majority of adults emerging as queens.

The honeybee appears unique among insects in that its DNA methylation system matches that of the human, where cytosines preceding guanines (CpG dinucleotides) are the targets of enzyme orthologs *AmDnmt1a and 1b* and *AmDnmt3*. The fraction of the *Apis* genome that is methylated is small (around three orders of magnitude smaller than for the human genome, comprising approximately 70,000 of the 60 million cytosines over approximately 6000 genes). There is a substantial technical advantage in studying a system analogous to human but greatly simplified and where effects on lifespan can be identified over a short time scale. Therefore the honeybee provides a compelling system for investigating epigenetic effects of diet on lifespan.

Regulation of mTOR and autophagy by lysosomal positioning

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A signal transduction pathway called mammalian target of rapamycin (mTOR) is an important sensor of cellular environment such as nutrients, general energy levels and oxygen. mTOR controls both protein synthesis and catabolism (mainly degradation pathway autophagy) and is deregulated in ageing and age-related diseases. Viktor Korolchuk from Newcastle University described a novel mechanism regulating the activity of mTOR and autophagy. In the model presented, nutrients appear to affect intracellular pH which, in turn, controls localisation of lysosomes. As the cytoplasmic surface of these degradative organelles is also the site of mTOR activation, proximity of lysosomes to the plasma membrane regulates the extent of mTOR induction by upstream signals generated by plasma membrane receptors. In addition, lysosomal positioning also appears to play a role in autophagy regulation by controlling both the synthesis and degradation of autophagic vesicles. These findings will help to devise new approaches to control mTOR and autophagy, which may be beneficial for the treatment of age-related diseases and for the extension of healthspan.

Mitochondrial translation activators modulate yeast longevity through regulation of nuclear silencing

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The role of mitochondria during aging has been studied extensively, essentially because mitochondria are the main damage generator in the cell due to the production of reactive oxygen species (ROS) during respiration. Furthermore, there are many mitochondrial mutations that cause a life span extension, which is frequently explained because low respiration activity in mitochondria decreases ROS production and that slows down the rate of aging. However, the classical theory of aging due to the harming nature of accumulating ROS is nowadays widely questioned, rising the possibility that mitochondria might regulate aging process by other mechanisms non associated with ROS generation.

We decided to study the relationship between mitochondria and aging in yeast due to the capacity of this organism to grow using fermentation, regardless non-respiratory mitochondria. We found, in fact, that a null mutant in the *SOV1* gene can extend life span independently of respiration and without varying the normal wild type ROS levels. Interestingly, a mutation in *SOV1*, which codifies for a mitochondrial protein necessary for the translation of the mitochondrial encoded gene *VAR1*, enhances nuclear silencing at the rDNA loci and extend life span in a *SIR2* dependent way.

SOV1 shares function with a group of protein in charge of activating the translation of specific mitochondrial-encoded genes. We have

found that many of these genes modulate life span in a *SOV1* fashion indicating that these mitochondrial proteins can regulate aging by increasing the levels of nuclear silencing. Two of those proteins, *CBS1* and *CBS2* are essential to translate mitochondrial cytochrome B, however only the mutant on *CBS1* extends life span and enhances silencing, and moreover, this phenotype requires the presence of the wild type copy of *CBS2*. We found that the protein Cbs2p is located both in mitochondria and in the nucleus, suggesting that these mitochondrial functions might regulate aging by interacting with some nuclear located partners. All together, these results indicate that mitochondria can in fact regulate aging by other means that a side effect of the respiratory activity.

Impact of Methionine Restriction and Atenolol treatment on mitochondrial oxidative stress in relation to longevity

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Comparative studies have clarified the only two known factors that correlate with maximum longevity and aging rate of mammals and birds: the mitochondrial rate of ROS production (mitROS) and the degree of fatty acid unsaturation (double bond index, DBI) of the cellular membranes in the main vital organs. Both are lower in long-lived than in short-lived animal species. The first of these two factors also decreases in dietary restriction (DR), the best known manipulation that increases longevity. We have clarified that not only DR lowers mitROS and oxidative damage to mtDNA. They are also lowered to the same extent by restricting the dietary intake of proteins or even of a single nutrient, the aminoacid methionine. These three dietary manipulations increase maximum longevity in mammals. Studies from our laboratory have shown that 40% Methionine Restricion decreases mitROS production and oxidative damage in heart, kidney, brain and liver mitochondria and we obtain the same results even when this manipulation is implemented at advance age in Wistar Rats. Methionine could be the only diet component responsible for the decrease in mitROS generation and oxidative stress, and likely for part of the longevity extension in DR. On the other hand we have found that blocking the 1 adrenergic receptors with atenolol decreases the second longevity-associated factor (the DBI) in mice through the AC/cAMP/PKA/ERK signalling pathway. Previously, Yan and co-workers had shown that mice knocked out for adenylyl cyclase-5 gene have delayed bone and heart aging and increased maximum longevity. Regarding to our results with atenolol, the longevity extension of AC5 KO mice could be due in part to a decrease in fatty acid unsaturation, lipid peroxidation and an increase of p-ERK signalling.

Eccentric exercise, ageing and muscular steadiness

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Introduction

Older adults experience a decline in muscle strength and steadiness, decreasing quality of life and increasing risk for falls. Adaptations by eccentric endurance exercise (i.e. downhill walking) maybe the ideal exercise modality to preserve muscle strength and steadiness in older adults. This is due to the resultant muscle injury; and inherently lower energy demand, that many conventional exercise interventions do not promote. The aim of the current study was to determine, in older adults, the effects of concentric (level treadmill walking) and eccentric endurance exercise (downhill treadmill walking) on maximal and submaximal sustained isometric contractions of *m.quadriceps femoris* (QF) with electromyographic recordings of the *m.vastus lateralis* (VL) and functional mobility.

Methods

18 healthy older adults (age: 67±4, body mass: 75±14 kg) completed 12 weeks of level treadmill walking (LW, 0%, n= 8) or downhill treadmill walking (DW, -10%, n=10) (30 min, 3 d·wk-1) at a self-selected walking speed (SSWS, re-adjusted in week 4 and 8). Maximal voluntary isometric force (MVIF) of QF and EMG of VL; 5-repetition sit-to-stand (5-RSTS), timed up-and-go (TUG) were measured at baseline, 4, 8 and 12 weeks. Steadiness of submaximal (5, 10 and

20%) isometric contractions (i.e. coefficient of variation of the force signal, CV)] of QF and EMG of VL was measured at baseline and 12 weeks. A two way repeated measures ANOVA with post-hoc pre-planned t-tests were used for data analysis (P<0.05).

Results

SSWS was similar for both groups and increased from 1.18±0.11 to 1.53±0.09 m·s-1 (LW) and 1.26±0.16 to 1.61±0.12 m·s-1 (DW) (P<0.01). Improvements in MWS, 5-RSTS and TUG were similar (P<0.01). MWS (baseline LW: 2.39±0.38 m·s-1, DW: 2.40±0.33 m·s-1) improved by 22 and 23%. 5-RSTS (baseline LW: 8.50±1.19s, DW: 8.54±1.52s) improved by 32 and 34%. TUG (baseline LW: 5.58±0.51s, DW: 5.46±0.89s) improved by 22%.Baseline MVIF of LW (340±112N) and DW (368±128N) increased equally by 14±6 and 5±6% (P<0.05). Steadiness at 5%MVIF improved following 12 weeks of LW (baseline: 0.04±0.01; 12 wk: 0.03±0.01) and DW (baseline: 0.04±0.02; 12 wk: 0.03±0.01 (P<0.05). EMG root mean square of VL during MVIF increased by 38% (P<0.05) following 12 weeks but only in the level walking group.

Discussion

Regular level and downhill treadmill walking at a self-selected walking speed resulted in substantial improvements in functional performance. It also appears to be at a sufficient intensity to improve the ability to produce maximal voluntary isometric force of *m.quadriceps femoris*. For level walking, this may be explained by an increase in neural activation whereas muscle hypertrophy and coordination may explain the changes after regular downhill walking (Sipilä et al., 1996). The improvement in steadiness at 5%MVIF may be due to a change in discharge rate variability (Laidlaw et al., 2000). Improvements in functional performance and ability to produce maximal isometric force and isometric steadiness, of submaximal isometric contractions by regular level and downhill treadmill walking at self-selected walking speed, may reduce the risk for falls in older adults.

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Age-related stiffening of the extracellular matrix

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The mechanical properties of dynamic tissues such as blood vessels, lungs, skin and cartilage are defined primarily by an extracellular matrix (ECM) in which fibrillar collagens and proteoglycans resist tensile and compressive forces respectively and the elastic fibre system confers resilience. Age-related changes in tissue stiffness and resilience are a key determinant of both human mortality (as a consequence of heart failure, stroke and acute respiratory infections) and morbidity (for example as a result of non-healing ulcers and low-back pain). However, whilst it is well established that ageing tissues undergo multiple changes in structure and composition, to date it has been necessary to infer a causal link between remodelling of specific components and tissue stiffening. In order to address this methodological limitation we have applied an adapted form of scanning acoustic microscopy to localise stiffening in the ageing sheep aorta to collagen-rich interlamellar regions within the medial layer (Graham et al., Mech. Ageing Dev. 2011;132:459-467). This technique has the potential to identify the key molecular targets of age related stiffening in diverse tissues. In addition, the causative mechanisms which drive age-related tissue remodelling are also poorly defined. We have previously shown that exposure to ultraviolet radiation (UVR) in vitro can mediate the differential degradation of proteins which are rich in UV-absorbing amino acids (Sherratt et al., *J. Pathol.* 2010;222:32-40). We have now demonstrated that reactive oxygen species (ROS), whether generated by UVR exposure or exogenously applied, can also mediate the differential degradation of long lived ECM proteins in vitro. We suggest therefore, that in the absence of mechanisms to detect and repair extracellular oxidative damage, ROS may play a key role in aberrant remodelling of mechanically active tissues.

Longevity-associated genes: from flies to humans

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The concept of longevity associated genes received serious support after a review by Sinclair and Guarente [1]. Many genes have been associated with longevity. In our laboratory, we have studied some of these. For instance we could attribute the increased longevity in females to the estrogen- dependent over-expression of Mn-SOD and Glutathione peroxidase [2]. Other genes that we have shown to be involved in longevity are p53 [3]; p16/Arf (4) RAS-Grf [5] and telomerase [6].
We have observed that, in humans, oxidative stress is associated more with frailty than with age itself. An obvious approach is to find physiological, nutritional or pharmacological intervention to promote the activity of longevity associated genes. Exercise is a paradigmatic intervention to promote such genes [7]
We have performed the whole mRNAi-ome and correlated it with the transcritome in centenarians (100 ± 2), octogenarians (80-85) and young persons of the area of Alzira (Valencia, Spain). Our conclusions are:

1.- The Principal Component Analysis (PCA) of the mRNAi ome of centenarians is remarkably similar to that of young persons and different from octogenarians
2.- Centenarians up-regulate the expression of mRNAi when compared with young persons. Octogenarians down regulated it. This shows that transcription in centenarians is much more regulated than in octogenarians.

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In vivo Characterization of Neuronal Ubiquitin Pathways: a starting point to understand ubiquitin-related neuronal diseases

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Post-translational ubiquitin conjugation to proteins is well documented to be involved in many cellular processes, from cell division, through development to neuronal function. Of particular interest to the ageing European population, ubiquitin appears to be involved in several neurodegenerative diseases. To date, nearly all of the work to understand the basis of ubiquitination has been performed in yeast and cancer cells. The same applies to ubiquitination proteomic studies, and hence most ubiquitin substrates have been identified in non-neuronal tissue. We recently developed a novel approach to isolate ubiquitinated substrates from neuronal tissue within a multicellular organism, which works very successfully in *Drosophila* embryonic neurons. Our aim is to first extend this approach to the *Drosophila* adult brain. In addition to characterizing the ubiquitin landscape in healthy individuals of various ages, we are applying our approach to a number of available neurological disease models known to be ubiquitin-related. In order to come closer to understanding human disease, once we have fully optimized our approach in Drosophila, we have now created transgenic mice on which we can deploy this same strategy. Similarly as with flies, we will first characterize the physiological ubiquitination landscape, and how it changes with ageing. Later, in combination with available mice models of neurodegenerative conditions, we will try to understand how the involvement of ubiquitination is significant to those diseases, in order to identify targets for future therapeutic approaches.

Osteoporosis: Evaluation of genetic polymorphisms

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Introduction

The prevalence of osteoporosis increases with age due to a disequilibrium between bone formation/resorption during the bone remodeling process. Several factors are involved, one of the most important is the presence of family antecedents of osteoporosis and fractures.

Objectives

To assess the genetic polymorphisms possible contribution to the bone mass loss increased risk measured by bone densitometry and the fractures development in transplant patients and postmenopausal women.

Material and methods

107 kidney transplant patients from the University Hospital Virgen de la Arrixaca (71 male, 33 female) and 224 menopausal women from the University Clinic of Navarra. We have done the polymorphisms genotyping of type 1 collagen (Col1A1 Sp1), calcitonin receptor (CTR-AluI), vitamin D receptor (VDR-FokI, VDR-BsmI) and estrogen receptor (ESR-XbaI, ESR-PvuII) genes (Clinical Arrays® MetaBone by Genomica) and the measurement of the bone mineral density (BMD) by Dual Energy X-ray Absortiometry (DEXA) in lumbar spine (LS) and femoral neck (FN): baseline measurement in menopausal women and pre-transplantation, 6 months, 1 year and 2 years measurements in kidney transplant patients, obtaining T-score and Z-score values.

Results

6 months after transplant a BMD decrease was observed in LS ($p<0.05$) and FN ($p<0.001$). Patients with "BB" genotype of VDR-BsmI gene have less LS BMD in the pretransplant phase ($p<0.05$), but they had a smaller BMD decrease 6 months after the transplant. Patients with "xx" genotype of ESR1X gene have lower FN BMD 6 months after transplant ($p<0.007$) and one year. 6 months after transplant the number of patients with a decrease in FN BMD below the 75th percentile of the sample is lower in patients who have the "PP" genotype of the ESR1P-PvuII gene ($p=0.02$), as well as the "ff" genotype of the VDR-FokI gene ($p=0.02$). There is a relationship between Col1A1 Sp1 and ESR1X polymorphisms: Of the patients with the "ss" genotype, 80 % are "xx". OR of osteopenia/osteoporosis: The "xx" genotype of the ESR1X gene is a variable predictor of having femoral neck BMD below the 50th percentile after 6 months (OR=3.069). In postmenopusal women, patients with the "ss" genotype of Col1A1 have less FN BMD, 80% of women with "BB" genotype have osteopenia, women with the "xx" genotype have fewer years of fertile life and the presence of the VDR-BB polymorphism involves 2.59 times greater odds of being affected by osteopenia/osteoporosis.

Conclusión

Genotyping based on extended panels of several polymorphisms might identify groups of people at high risk of osteoporosis, with fewer costs and decades before BMD begins to decrease.

DNA polymerase μ, a member of the NHEJ pathway, plays a relevant role in murine ageing

Antonio Bernad
CNIC, Madrid

Multiple alterations of organism physiology contribute to the aging process, but modification of the DNA metabolism/repair equilibrium seems to play a prominent role. DNA polymerase μ (Polμ) is a novel member of the PolX family, structurally related with TdT but widely expressed in most tissues at low levels. Pol μ has been demonstrated to participate in NHEJ repair with a special involvement in repair of DSBs with 3´protuding ends and low microhomology.

Analysis of the Polμ KO model demonstrated a mild increased in endogenous damage (-H2AX staining) and augmented radiosensivity, both in whole animal and purified cells. In addition, a significant alteration in hematopoietic

homeostasis was demonstrated. Those global phenotypes were consistent with the elimination of an ancillary DNA repair function. Surprisingly, Polμ KO colony demonstrated a significant (32%) lifespan extension, and Polμ KO old animals preserve learning capacities at ages in which the wt controls are clearly declining. We discarded that Polμ KO were more resistant to spontaneous or induced cancer. Molecular analysis of liver demonstrated a very significant reduction of p53-regulated genes presenting a net reduction (12-fold) in apoptotic cells. Finally, all the molecular hallmarks can be integrated in a working hypothesis inferring that the moderate reduction in NHEJ activity promotes a compensatory mechanism that augments the participation of homologous recombination (HR). This conform a DNA repair machinery, less efficient but more accurate, that modulate organismal aging.

Identification and characterisation of new models for presbycusis

Prashanthini Shanthakumar,
Medical Research Council Harwell

Presbycusis or age-related hearing loss is a very significant health and social burden on the ageing population. It affects the elderly population with high prevalence, and both genetic and environmental factors influence its onset and progression. Approximately 60% of adults over 65 years of age have some degree of hearing loss, with reduced sensitivity to sound and to speech perception. To date, little is known about the underlying genetics of presbycusis, despite considerable efforts in performing genome-wide association studies (GWAS). In mice, most age-related hearing loss research has focused on the variation between inbred strains. Towards this, eleven loci have been identified as causing age-related hearing loss in certain inbred strains. Currently, the underlying genetic lesion for three loci have been identified. The trait age-related hearing loss *(ahl1)* has been determined to be a hypomorphic allele of the cadherin 23 gene and has been described as an accelerating allele of the disease. Other loci such as ahl5 *(Gipc3)* and ahl8 *(Fscn2)* have also been implicated in age-related auditory decline.

At MRC Harwell, we have embarked on a major ENU mutagenesis programme, which is a phenotype-driven mutagenesis approach, to recover novel mouse models of diseases of aging. Under this programme, male mice are injected with ENU and having regained fertility are bred to produce mutant offspring which can be screened for diseases of interest. The Deafness Model Discovery team is taking advantage of this screen to identify and characterise mouse models of presbycusis. G3 pedigrees, of ~100 mice, will be aged and undergo phenotyping across a wide range of disease areas, including sensorineural. We will conduct recurrent auditory phenotyping at several defined time points, consisting of Clickbox (20 kHz tone, 90dB SPL) at 3, 6, 9 and 12 months of age, and Auditory-Evoked Brainstem Response (click, 8, 16, 32 kHz) at 3 and 9 months.

The first pedigree of interest was identified at 9 months of age – MP90. Several mice from this pedigree have elevated ABR thresholds, compared to littermates and other G3 mice when tested at 9 months of age, which is most pronounced at the highest frequency tested (32 kHz). Initial mapping was undertaken on 9 animals and the results obtained were inconclusive and did not highlight a specific region. However, there were three potential intervals on interest on chromosomes 4, 13 and 19. Further mapping on additional animals has confirmed a region of interest on chromosome 4: 145785696-tel. DNA from one affected mouse was also sent for next generation sequencing. Initial sequence analysis identified no coding or splice site changes, however, there are sequencing gaps which are currently being analysed. Initial SEM analysis showed some dysmorphology of the stereocilia of the affected animals in comparison to the unaffected animals. However, further analysis is required on more affected animals.

The second interesting pedigree was identified at 3 months of age – MPC96. Several mice from this pedigree also exhibited elevated ABR thresholds, compared to littermates and other G3 mice when tested at 3 months of age. The severity of hearing loss is shown to be most pronounced at the highest frequency tested (32 kHz) which is what is commonly observed in presbycusis patients. The mice were aged to determine whether

the hearing loss gets progressively worse and early data has shown that there is a progression. DNA from affected and unaffected mice were sent for mapping and this has identified a candidate region on chromosome 2: 51425686-114161459Furthermore, the original G1 male has been sent for sequencing and early results indicate two potential high confidence changes in our candidate region.

Subsequently, as interesting mutants are identified, genome mapping and next generation sequencing will be employed to identify the underlying genetic lesion. The power of this approach is that no a *priori* assumptions are made about the genetic loci underlying the disease and hence is a powerful approach for discovering novel genes and pathways. Characterisation of these models will help to elucidate the genetics of age-related hearing loss.

Antioxidants and cell function

Patricia Santofimia,
Facultad de Veterinaria de Cáceres, Universidad de Extremadura

Alcohol consumption has long been associated with cell damage. Ethanol's action might be mainly mediated by a slowing down of the activity of SERCA and PMCA in mouse pancreatic acinar cells, which will lead to increased levels of [Ca^{2+}]c after CCK-8 stimulation. The actions of ethanol on CCK-8-stimulation of cells create a situation potentially leading to Ca^{2+} overload, which is a common pathological precursor that mediates pancreatitis.

Ethanol induces generation of ROS by a Ca^{2+}-dependent mechanism and reduces CCK-8-evoked amylase secretion in exocrine pancreatic cells. Furthermore, Ca^{2+} influx was increased in the presence of ethanol, and inhibition of ethanol metabolization, or preincubation of cells in the presence of the antioxidant cinnamtannin B-1, reverted ethanol evoked effects.

The beneficial effects of cinnamtannin B-1 appear to be mediated by reducing the intracellular Ca^{2+} overload and intracellular accumulation of digestive enzymes evoked by reactive oxygen species.

In rat hippocampal astrocytes, ethanol leads to a decrease in Ca^{2+} mobilization in response to stimulation with kainate. This effect might be related to a decrease in kainate-induced glutamate secretion. The effect of ethanol may involve oxidative metabolization, and is counteracted by the antioxidant cinnamtannin B-1, suggesting a dependence of ethanol effects on ROS generation.

Finally, melatonin reduces viability of tumor AR42J cells via its action on mitochondrial activity and caspase-3 activation. Resveratrol releases Ca^{2+} from intracellular stores, most probably from the endoplasmic reticulum, and reduces AR42J cells viability by activation of c-Jun N-terminal kinase.

COLLABORATIONS

Sarah Barnes:

There is a possible collaboration between myself and Inés Sánchez-Román from Universidad Complutense de Madrid to look at the liver of old aged methionine restricted rats and controls to see if *Cidea* expression is conserved between rodents and if it is, does the reduction in methionine reduce the expression of *Cidea*)

Antonio Bernad:

Plausible collaborations with Richard Faragher, University of Brighton on Evaluation of senescence in Polμ KO aging phenotype and with José Viña, Universidad de Valencia on Evaluation of oxidative stress in Polμ KO aging phenotype

James Brown:

I am happy to say that I am now in talks with Professor Plácido Navas, Professor José Viña and Professor Luis García Segura about collaborating on several research areas. I will be visiting Seville again in May to meet with Professor Navas

Luis García Segura:

Possible collaborations with Matthew Hardman exploring possible common signaling mechanisms involved in skin wound healing and neural tissue reorganization after traumatic brain injury, based on previous results revealing an interaction of the signaling of IGF-I receptor and estrogen receptors in both tissues and with James Brown, Aston University, Birmingham on Studies on the interaction of estrogenic compounds and IGF-I receptor signaling on neuro-glia metabolism.

Matthew Hardman:

Collaboration with Luis García Segura exploring possible common signaling mechanisms involved in skin wound healing and neural tissue reorganization after traumatic brain injury, based on previous results revealing an interaction of the signaling of IGF-I receptor and estrogen receptors in both tissues.

Miriam Martínez:

We have establish a collaboration with Elena González-Rey's group, (Instituto de Parasitología y Biomedicina López Neira de Armilla) and my group on: "to assess levels of adrenomedullin and cortistatin neuropeptides in cerebrospinal fluid of patients with dementia (possibility of developing other central nervous system diseases) compared with healthy individuals"

Ugo Mayor:

Together with Viktor Korolchuk, from the Newcastle Institute for Ageing and Health, we are initiating a consortium for submitting an application to the FP7-HEALTH-2013 call which will be officially published in July this year.
The topic for the application is ageing (HEALTH.2013.2.1.1-1: Functional validation in animal and cellular models of genetic determinants of diseases and ageing processes), and the general theme would be the perturbation of ubiquitin homeostasis in ageing and age-related diseases.

We are assembling a line up of labs using cell models as well as Drosophila, C elegans and mice. We are contacting further groups in order to create a consortium of the right size. Furthermore, we already have a positive reply from at least two SMEs.

José Viña:

The meeting has been extremely useful and in fact, it has resulted in a line of cooperation between the laboratory of Ugo Mayor (Bilbao) and that of Viktor Korolchuk, from the Newcastle Institute for Ageing and Health. This may result in the initiation of a consortium for submitting an application to the FP7-HEALTH-2013 call which will be officially published in July this year.

Antonio Miranda:

Collaboration con Antonio Caballero on Evaluation of insulin levels in C. elegans trx-1 mutants

Michael Sherratt:

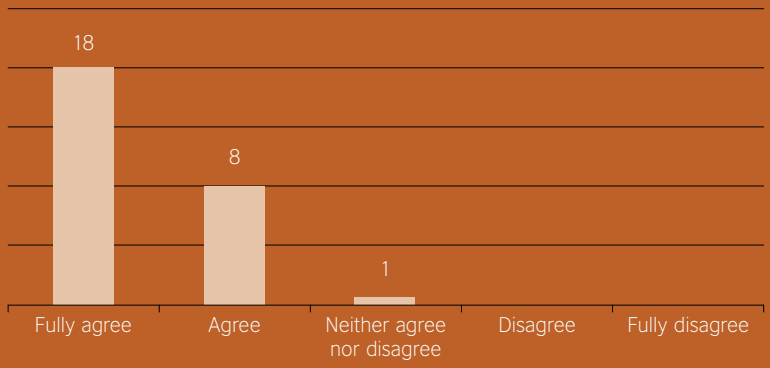
With regards to collaborations Antonio Miranda and I have started a discussion as to the potential common pathways which may contribute to stiffness in the nematode cuticle and human skin. I will also collaborate with David Kipling on more advanced bioinformatic analyses of amino acid composition.

Luisa Wakeling:

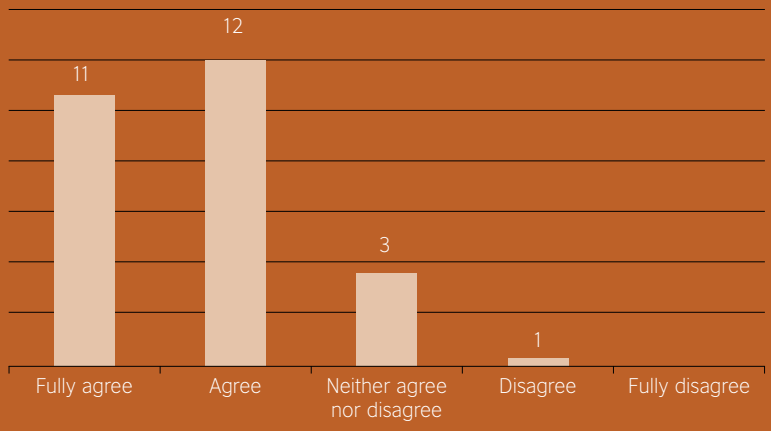
I was very interested in the work by Inés Sánchez-Román and her methionine restricted rats. A change in methionine of the bee diet is something that we intend to do once we have secured funding and if we are able to identify target genes that change because of this dietary manipulation, it would be very interesting to see if this "change" also happens in other organisms. I have not approached Ines directly about this but will in due course if we are successful with our funding application and subsequent experiments.

EVALUATION QUESTIONNAIRE

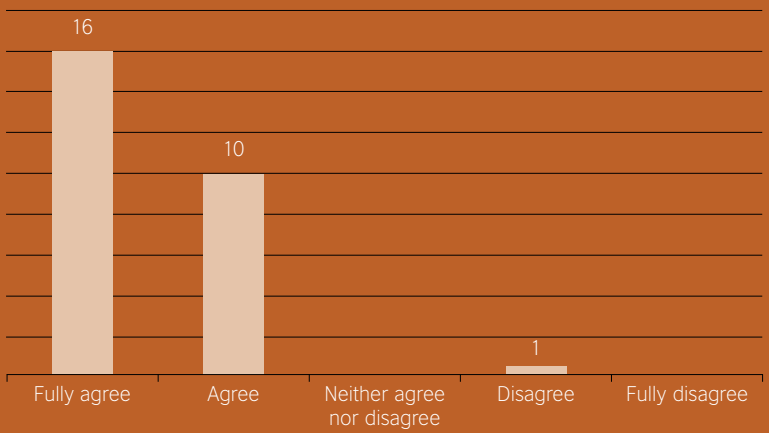
1. It was a well balanced programme



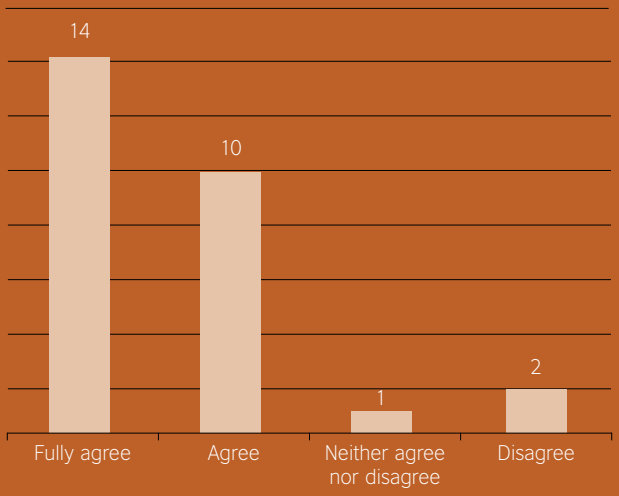
2. The discussion timing was right



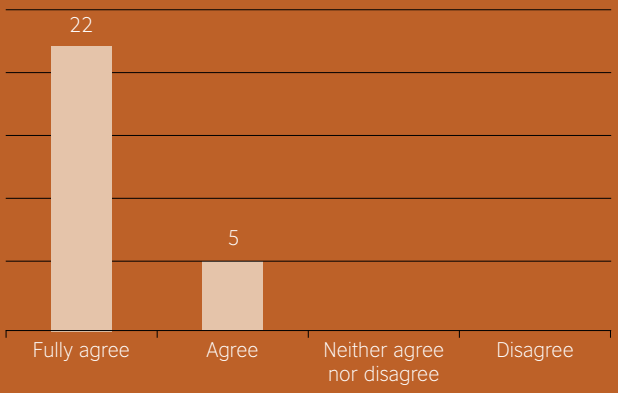
3. The length of the seminar was correct



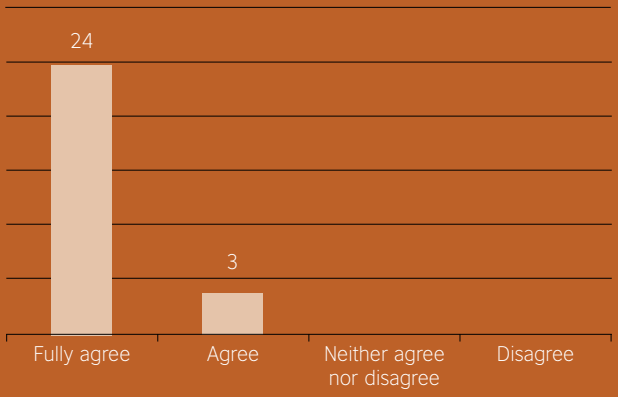
4. The mix of participants was right



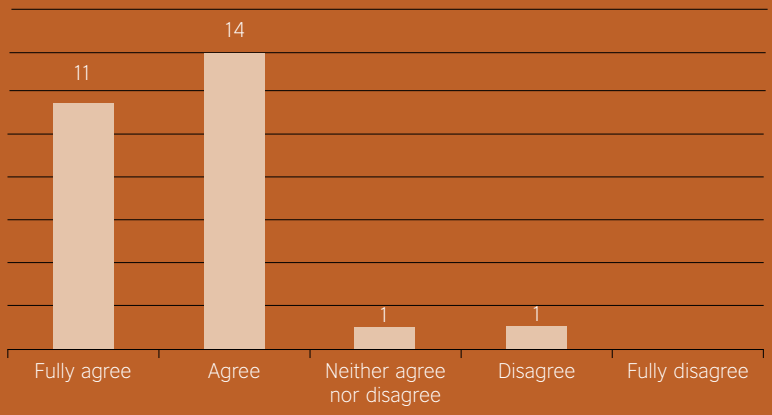
5. The conference facilities were good



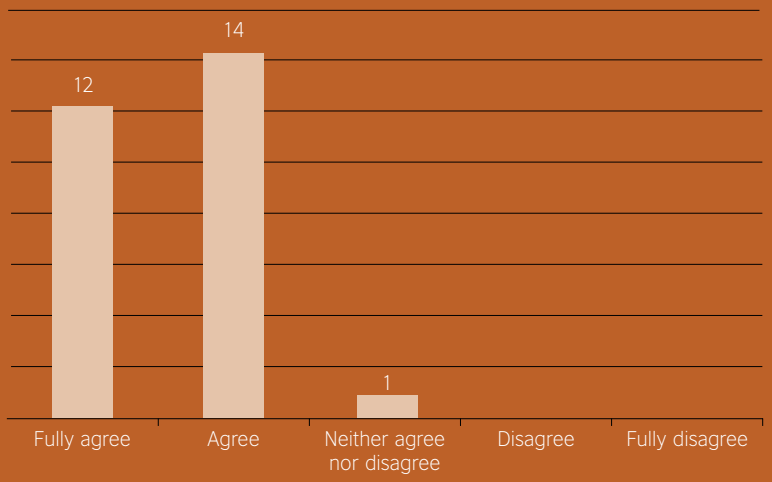
6. The standard of accommodation was good



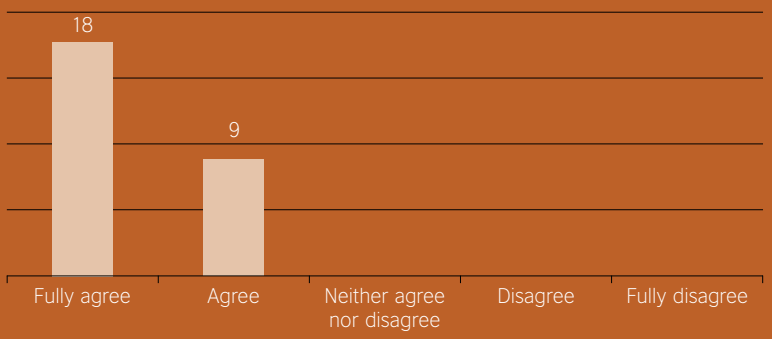
7. The food was good



8. The seminar meet my expectations?



9. Overall, this was a high quality seminar



10. Overall, how would you rate the quality of our services?

Results of Event Evaluation Questionnaires

General data

Number of participants	31
Number of questionnaires received	27
Percentage	87%

GENERAL COMMENTS

A very useful seminar. (Prashanthini Shanthakumar)

This was an extremely well organised and informative event. (Viktor Korolchuk)

Only some specific round tables on selected hot topics could improve the workshop. (Antonio Bernad)

This workshop has been a valuable experience. (Hayley Lees)

This workshop has been especially useful to establish contacts, to meet senior researchers and to make friends. (Rubén Corpas)

An enjoyable and scientifically successful conference. (Lynne Cox)

A great experience. (Yila de la Guardia)

The meeting was excellent. (Matthew Hardman)

A great experience. The content of the workshop was of a very high level. I've learnt a lot. I have interacted with many people and I hope to start collaborations soon. Thank you for giving me this opportunity. (Elena González-Rey)

A great workshop. (Luisa Wakeling)

Thank you for organising such an excellent meeting not only for the scientific topics discussed but also for the information on the opportunities of collaborative work between both our countries. (Antonio Miranda)

ACKNOWLEDGEMENTS

We would like to thank the Consejo Superior de Investigaciones Científicas and the British Council for organizing this excellent and thoroughly enjoyable scientific meeting. The event has provided a great deal of opportunities for dialogue and discussion whilst demonstrating the strength of our young scientists. We would like to emphasize in particular the hard work and kindness of Belen Fortea of the British Council. Her careful continued support and efficiency made it possible the success of the workshop in Carmona not only from the scientific point of view but also providing a perfect frame for the necessary social interaction that this type of meetings pursue.


BRITISH COUNCIL
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"Biología del Envejecimiento"
"The Biology of Ageing"


Rod Pryde
Miguel Ferrer Baena