9th European Congress of Biogerontology

FACING CHALLENGES IN AN AGING WORLD

Centro Andaluz de Biología del Desarrollo
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INTRODUCTION

Guillermo López-Lluch

Departamento de Fisiología, Anatomía y Biología Celular, Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide CSIC, CIBERER-Instituto de Salud San Carlos III, Carretera de Utrera Km 1, 41013, Sevilla, Spain.

It is clear that the amount of people reaching old age is increasing worldwide. During last decades, the percentage of population aged more than 65 has been increasing. In fact, United Nations Organization reports indicate that the percentage of population aged more than 60 years was around a 10% in 1999 in the world but this increase will reach the 20% in 2050. Part of this effect is due to the increase lifespan of humans during last decades. It has been calculated that between 2045 and 2050 the lifespan of people aged 80 years will be around 10 years. It means that the number of nonagenarians and even centenarians will increase soon (United Nations Department of Economics and Social Affairs, World Population Ageing 2013). Currently Europe has reached a 20% of population aged more than 60 years and in 2050 this proportion will be around 30%. Taken into consideration the needs of this population, the sustainability of social and health systems needs to be revised.

Several initiatives have been proposed to increase health and independency in elderly people. Increasing healthspan in aged population is one of the most important challenges in the near future. For example, the program EIP-AHA (Europa Innovation Partnership on Active and Healthy Ageing) of the European Union pursues three main objectives involved in the increase in health span in European citizens.

It is widely known that life habits deeply affect the capacity of cells, organs and tissues. Sedentary life habits and the increasing consume of saturated fatty acids is severely affecting the risk to suffer cardiovascular diseases, obesity, diabetes and metabolic diseases, all of them associated to chronic treatment during ageing.

For this reason, the importance of the Biogerontology, the part of the science that study the biological aspects of ageing, is increasing since without

knowing how ageing occurs and if it can be modulated we cannot design plans to improve healthspan in elderly population.

In this book we offer a compendium of the lectures and abstracts shown in the 9th European Congress of Biogerontology celebrated in Seville the 16th-18th October, 2014. I hope you will find a wide landscape of the different field involved in the research on ageing.

Two conferences offered a perspective of ageing in humans from a translational point of view. Diana Kuh (UCL, London) and Luigi Ferrucci (NIA, Bethesda, USA). Sessions covered the different aspects of biogerontology. We treat the health challenges that Europe must face in the near future.

Lectures treated different aspects of ageing as health and healthy ageing (S. Rattan), peptides, genome and ageing (V. Khavinson) and the finding of biomarkers in ageing (A. Bürkle).

Another session covered the new findings about stem cells and regenerative medicine and their applications in ageing. The aspects of cellular senescence in tissue remodeling (D. Muñoz), the modifications of biology in stem cells from human adipose tissue (W. Zwerschke) and the aspects of skin remodeling (V. Fraifeld) were covered.

The importance of metabolism in ageing is clear. A great body of research is being performed in order to understand how metabolism is affecting ageing and viceversa. This aspect was covered showing the influence of fat in the diet in longevity in calorie restricted animals (J. Ramsey), the finding of a unifying biochemical mechanism of metabolism and ageing (S. Sollot), and the importance of adipose tissue in ageing (J. Brown). Oral communication presented by López-Domínguez showed the gene expression changes affected by dietary fat in calorie restricted animals whereas A. Sanz presented the importance of ROS signals in the mitochondrial function in D. melanogaster.

Antioxidants are considered important factors in ageing. In fact, one of the main theories to explain ageing is based on the oxidative damage accumulation due to a higher amount of reactive oxygen species. This aspect was covered by the lectures of M. Bernier on the role of cytochrome B5-reductase, an antioxidant enzyme in membranes, and longevity. The role of other oxidoreductase, NQO1, in antioxidant protection and metabolic regulation was the subject of the lecture showed by R. De Cabo. Other aspects were treated in oral and poster communications. Among then, E. Jansen showed

the effect of antioxidant supplementation in different human age groups and A. Lloret treated the relationship between the risk to suffer Alzheimer's disease by reductive stress.

Impairment of the muscle and nervous system activity was treated in a specific section. The importance of rodent models in the study of neuromuscular physiology was shown by A. Gruart. The effect of age on signals affecting muscle activity was treated by the lecture of A. Guadalupe-Grau. The importance of physical exercise in the maintenance of synaptic plasticity in a model of accelerated senescence was also found as oral communication (J.C. Lopez-Ramos).

Genes have been considered important factors in ageing progression. To this, the modifications that affect the structure and function of chromatin and then, the regulation at the DNA level are building a new network of interactions in many physiological aspects including ageing. These aspects were covered in different lectures. The specific regulation of gene expression in centenarians (J. Viña) and the genomic predictors of a higher longevity (G. Lehmann) were interesting lectures in this field. These were complemented by oral communications showing the role of regulatory signaling pathways in aging models such as *C. elegans* (Y. Budovskaya), the regulation of methyl-residues in some genes and its influence in ageing (G. Passarino), the modifications in the gene expression patterns during aging in humans (A. Viñuela), and the effect of life-style factors in nonagenarians from a Belfast cohort (M. Rea).

Inmunosenescence is another important factor that severely affects ageing progression and ageing-related diseases. The importance of the immune system in ageing was shown by G. Pawelec. R. Solana showed the role of the Natural Killer cells in ageing and the relationship of its activity and different populations with the infection by citomegalovirus. The relationship of ageing and the communication of neuronal and immune systems was shown as an oral communication (I. Martinez de Toda).

Finally, a session covered the importance of coenzyme Q in ageing. This session was sponsored by the International Coenzyme Q10 association. Coenzyme Q10 is a lipid compound involved in the activity of the electron transport chain in mitochondria and also in the activity of membrane-linked oxidoreductases important in antioxidant protection and metabolic regulation. Different aspects of the activity of Coenzyme Q10 in ageing were treated in this section. K. Higuchi showed the activation of mitochondrial

function and the improvement of senescence by reduced coenzyme Q10 in a murine model of accelerated senescence. The importance of coenzyme Q10 in cardiovascular system protection was treated by I. Hargreaves. The link of coenzyme Q biosynthesis and mitochondrial turnover was treated in the lecture of C. Santos-Ocaña and the adaptation of the biosynthesis machinery affecting coenzyme Q synthesis to the level of calorie intake was shown by J. Villalba.

All these sections were also accompanied by different posters that have been associated to the different sections in this book. The reader of this book will then find a picture of the different fields included in the study of the biological ageing in different models from molecular to epidemiological aspects. I hope this information will be useful to, at least, understand how complex in the Biogerontology field and how much effort we have to do to increase longevity and, more importantly, health span during ageing.

Dedicated to my grandparents and parents. I hope I received some of the good genes to reach an active ageing specially from my grandmother Transito Moreno Fernández (died at 94 years), a little but strong woman.

CONFERENCES

INAUGURATING CONFERENCE

A life course approach to healthy ageing: evidence from the MRC National Survey of Health and Development and other UK cohort studies

Kuh. D[1]

Research on factors that determine healthy ageing is a priority of governments and funding agencies to inform strategies for reducing societal and individual costs of an ageing population. This field lacks an agreed conceptual framework and has spawned many definitions of healthy ageing. This presentation discusses how healthy ageing may be conceptualised and investigated within a life course framework that acknowledges the growing evidence that social, psychological and biological factors from early life onwards affect the chance of healthy ageing. Research findings will be presented from two sources: from the MRC National Survey of Health and Development (NSHD), a nationally representative sample of British men and women followed since their birth in March 1946, so far for 68 years[2]; and from cross cohort studies which attempt to replicate these findings to strengthen causal inference and policy relevance. Diana has been at the forefront of the development of life course epidemiology.[3, 4, 5]

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2 Kuh D, Pierce M, Adams et al. Cohort profile: updating the cohort profile for the MRC National Survey of Health and Development: a new clinic-based data collection for ageing research. International Journal of Epidemiology 2011; Feb; 40(1):e1-9.

3 Kuh D, Ben-Shlomo Y (Eds). A life course approach to chronic disease epidemiology: tracing the origins of ill-health from early to adult life. 2nd edition. Oxford University Press 2004.

4 Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges, and interdisciplinary perspectives. International Journal of Epidemiology 2002; 31:285-93.

5 Ben-Shlomo Y, Mishra G, Kuh D. Life Course Epidemiology in Handbook of Epidemiology, 2nd edition. Springer. 2014.

Her latest co-edited book *A life course approach to healthy ageing*[1] integrates conceptual frameworks and models from life course epidemiology and ageing research, and reviews the evidence linking factors operating from early life to optimal adult functioning at the individual, body system and cellular levels, and to wellbeing.

Diana is the Director of the NSHD and of the MRC University Unit for Lifelong Health and Ageing at UCL.

1 Kuh D, Cooper R, Hardy R, Richards M, Ben-Shlomo Y (Eds). A life course approach to healthy ageing. Oxford University Press, 2014.

CLOSING CONFERENCE

Research on aging: toward a translational perspective

Ferrucci. L[1]

Over the last few years research on the biology of aging has made unprecedented progress. Up to 20 years ago, the appearance of a scientific report on the biology of aging in one of the top rank biological journals was a rare event, almost considered a curiosity or a singularity. More recently, research on the biological mechanisms that underlay aging has grown into a critical branch of science. Research in cell and animal models of mechanisms that may affect aging or modulate its progression is now the main focus of entire laboratories and research institutions around the world. Candidate mechanisms include epigenetic modifications, DNA repair, cell senescence, telomere shortening, impaired proteostasis, impaired mitochondrial function and others. Unfortunately, very little of such new wealth of knowledge has been translated to better care for older persons and to the development of interventions that can slow down aging and prevent or delay the decline in health and physical function that is unavoidably associated with the aging process. Indeed, it is still unclear whether candidates aging mechanism and the development of the susceptibility phenotypes typical of aging are in any way related. Promoting aging well will require delaying major diseases and disabilities and their consequences. Medical research has been successful in controlling and preventing specific diseases in persons that were otherwise relatively healthy. However, as the population ages, such disease specific approaches start showing limitations and needs to be supplemented by a more global approach that takes into account measures of susceptibility and resilience that may strongly affect competing risks. As people live longer and develop susceptibility, they are more likely to fall victim to multiple diseases and preventing or treating one diseases is increasingly likely to result in an alternative cause of death or disability

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with limited gain in healthy or total life expectancy. This view is consistent with recent findings suggesting that unrelated morbidities cluster in the same individuals and that mild chronic inflammation and hyper-metabolism are strong independent predictors of multi-morbidity. Technology is now available that allows assessing candidate biological mechanisms of aging. At the same time, geriatricians, population scientists and gerontologists are developing robust measures of susceptibility and resilience that can be collected in clinical populations. In most cases, these research fields work as separate entities and are unlikely to collide. Progress will require biologists, gerontologists, population scientists and geriatricians to find a common language and start operating as multidisciplinary team.

SESSION 1
Health challenges in an ageing Europe

INVITED SPEAKERS

Health, homeodynamics and healthy ageing

BIOGERONTOLOGY-SPRINGER LECTURE

Rattan. S[1]

We want to maintain or enhance health, and we wish to achieve healthy ageing. But what exactly is health? Most of the biomedical research is still dominated and supported by disease-directed priorities. A change in thinking, approach and strategy is required to understand health, maintain health, improve the quality of life, extend the health-span and enhance public- and social-health. Ageing is not a disease; there are no ageing causing gerontogenes; and there is no "enemy within". Ageing occurs in spite of the presence of complex pathways of maintenance, repair and defence. The very act of living causes damage in our cells. A network of molecular, cellular and physiological maintenance and repair systems creates a "homeodynamic space" to tolerate, buffer and adapt against such damages. Ageing is the progressive shrinkage of the homeodynamic space, reduced resilience and increased vulnerability. An effective approach towards healthy ageing is that of hormesis, which is the positive relationship between mild stress and health. Conditions that induce hormesis are called hormetins, and are categorised as nutritional, physical and mental hormetins. Two challenging questions that need to be given high priority in biological ageing research are: (i) what is the functional relevance of various types of molecular damage that accumulate during ageing? And (ii) what are the biological determinants of health and homeodynamic space in terms of stress response profiles, damage control and tolerance, and adaptive abilities?

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Peptides, Genome, Ageing

Khavinson. VKh[1, 2, 3], Linkova. NS[1, 4], Tarnovskaya. SI[1]

Short peptides administration to experimental animals leads to increased average lifespan and decreases growth of spontaneous, induced and transplanted tumor. Di-, tri- and tetrapeptides influence on gene expression in different animal organs. Short peptides administration into transgenic mice suppresses the expression of gene HER–2/neu, which plays an important role in development and progression of mammary cancer. This effect correlates with reduction of adenocarcinoma size.

For the first time the mechanism of peptide geroprotective effect has been established as activator of chromatin in lymphocytes of elderly patients. The addition of AEDG peptide in human fibroblast cell culture leads to induction of telomerase activity and telomere elongation as compare to the control. This results in increase of cell division by 42.5%.

Application of peptides in elderly and old people leads to metabolism recover, increase of melatonin level, rehabilitation of the main physiological functions and reliable mortality decrease during the period of 15 years as compared to control. It was established that peptides interact with single- and double-stranded deoxyribooligonucleotides containing CG and CTG sequences. These sites are targets for cytosine DNA methylation in eukaryotes. Peptides selectively modulate *in vitro* influence of wheat endonucleases containing CNG or CG sites (WEN1 and WEN2) on DNA according to DNA methylation status. They also interact with histones H1-H6.

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Thus, short peptides interact with specific DNA sequences and histones leading to activation of mRNA transcription and translation of proteins involved in cell proliferation, differentiation and apoptosis. Peptides could epigenetically control cell functions through activation of matrix synthesis, which leads to homeostasis normalization and increased lifespan.

Biomarkers of human ageing. Results from the EU PF7 project MARK-AGE

Bürkle. A[1] for the MARK-AGE Consortium[2]

The rate of ageing in humans is not uniform, for various reasons. Age-related changes in body function or composition that could serve as a measure of "biological" age and predict the onset of age-related diseases and/or residual lifetime are termed "biomarkers of ageing". Many candidate biomarkers have been proposed but in all cases their variability in cross-sectional studies is considerable, and therefore no single measurement has so far proven to yield a useful biomarker of ageing on its own. The MARK-AGE Consortium (www.mark-age.eu) has therefore conducted a population study (3,300 subjects) aiming at the identification of a set of biomarkers of ageing that could serve as a measure of biological age. Two larger groups of subjects have been recruited, i.e. (i) randomly recruited age-stratified individuals from the general population covering the age range 35-74 years and (ii) subjects born from a long-living parent belonging to a family with long living sibling(s) already recruited in the framework of the GEHA project. For genetic reasons such individuals (termed GEHA offspring) are expected to age at a slower rate. They have been recruited together with their spouses as controls, thus allowing initial validation of the biomarkers identified. (iii) A small number of patients with progeroid syndromes have also included in the study. A wide range of candidate biomarkers were

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2 www.mark-age.eu

tested. Bioinformatic analyses have been performed to extract a robust set of biomarkers of human ageing from the large amounts of data generated. Data on the top 10 biomarkers will be shown.

ORAL COMMUNICATIONS

The human being possesses properties of potentially ageless being. What prevents realization of this possibility?

Khalyavkin. A[1, 2]

While anti-ageing researchers have made considerable advances in recent years, anti-ageing thinking, as a formal discipline, has yet to enter the mainstream. I believe that this is partially a consequence of insufficient attention to and an underestimating of the future social issues - both the negative and positive. Indeed, are we ready to be non-senescent now? We may say 'Yes' if it is a part of the scientific task. But we must say 'No' in a case if it will be a global social project. It is so since at present our society is too heterogeneous, too imperfect and too inconsistent for the realization of this possibility. Therefore the overcoming of social imperfectness is a top-priority challenge of our days. But what are the biological bases of our confidence in feasibility of non-senescence? The limitless replicative potential which somatic stem-like cells are thought to possess is clearly revealed by means of restoration of the stem cell supporting microenvironment/humoral background. We also know that the control system of a potentially non-senescent organism is able to sustain a physiological regimen of complete self-maintenance not in any circumstances but only within a certain range of changes in the total external conditions known as "environmental pressure". Outside this zone even potentially ageless *Hydras* start to experience a Gompertzian (exponential) ageing. The patterns of human mortality are also compatible with this view. But certainly we

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must have a reliable road map for the future harmonic society long before possible wide world realization of ageless individuals.

POSTERS

Older adults and food-drug interactions: self-medication and impact on nutritional status

Pinto Navarro. C[1], Rodríguez Griñolo. MR[2], Ortega de la Torre. MA[3]

A descriptive cross-sectional study was carried out in the Active Participation Centers in the towns of La Algaba and Sanlúcar la Mayor, both subordinated to the province of Seville, about self-medication and the potential impact on nutritional status in a sample of older people. 34 older people were interviewed. The average age was 71.4 years. It was found that 65% of participants self-medicate, of which 5% consume over-the-counter drugs or herbs to treat only the disease that affect them, 77% do it only if they have a different illness from the underlying disease and finally, 18% of participants who self-medicate do it for both treating a primary disease and to relieve other ailments. One of the main reasons for self-medication to treat a condition different from the underlying pathology is to relieve headache or muscle aches. The usual drugs and herbs in that case of self-medication were anti-inflammatories, such as acetaminophen and ibuprofen, and chamomile. Regarding nutritional status, 59% of the interviewed people are

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on normal weight according to the Body Mass Index (BMI), and 85% has a normal nutritional status, according to the Mini Nutritional Assessment (MNA) tool. In our study, there are no statistically significant differences among socio-demographic variables and self-medication status, although the profile of self-medication tendency was female widow, older than 80 years, and living alone. There is no relationship neither between nutritional status assessed by MNA tool or by Body Mass Index and the presence of self-medication.

Key words: Elderly. Seniors. Surveys. Self-medication. Food-drug interactions. Drug-nutrient interactions. Nutritional status. Prescribing medicines. Drug utilization.

Health and Quality of life in older adults: objectives and subjective indicators

Alarcón Rubio. D[1], Fernández Portero. C[1], Sánchez Medina. JA[1], García Amián. J[1]

The construct of quality of life and its operational definition, to a continuous controversy being a concept broad, ambiguous and devoid conceptual framework concept as it has prevailed in its path the lack of consensus on its definition (Birren Dieckmann, 1191; Bowling, 2005, Brown et al, 2003;. Fayers and Machin, 2007; Lawton, 1983; Moons, Buddts and De Geest, 2006, Nussbaum and Sen, 2002; Veenhoven, 2000; Walker, 2005b). The aim of this study was to analyze the relationship between various indicators of quality of life and health of over 65 years. The sample consists of 376 people over 65 years of Sevilla who collaborated with the research voluntarily. Measures of Quality of Life were evaluated by the Brief Quality of Life Quality of Life (CUBRECAVI) (Fernandez-Ballesteros & Zamarrón, 1996). The results indicate that the Quality of Life is a construct which is

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influenced by both objective and subjective indicators. This indicates the importance of assessing the construct of quality of life from a multidimensional point of view by including the objective aspects (dimensions of quality of life), a subjective dimension of valuation indicators, judgment or feeling, associated with the perception of the subject's own assessment issued within the state as Verdugo and Vincent (2004).

Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES)

Jansen. E[1] (on behalf CHANCES consortium)

Introduction.CHANCES[2] (Consortium on Health and Ageing: Network of Cohorts in Europe and the United States) is a collaborative large-scale integrating project funded by the European Commission within the Seventh Framework Programme. The CHANCES Consortium consists of 17 partners and 2 collaborators. The project, coordinated by the Hellenic Health Foundation in Athens, started in February 2010 and will end in January 2015.

Objectives. The CHANCES project aims at harmonizing data from existing major longitudinal studies for elderly. Focus is directed on four groups of chronic diseases which are major contributors to the burden of disease in the elderly: Cancer, Cardiovascular diseases and diabetes, Osteoporosis and fractures, Cognitive function and dementia disorders. The expected results from CHANCES are to estimate the prevalence, incidence of and cause-specific mortality from these conditions, and identify possible lifestyle, socioeconomic, and genetic determinants and biomarkers.

Results. Fifteen cohort studies participate in CHANCES with 686,475 elderly (and approximately 125,000 deaths), from twenty three European

1 Centre for Health Protection, National Institute for Public Health and the Environment. PO Box 1, 3720 BA Bilthoven, the Netherlands. e-mail: eugene.jansen@rivm.nl

2 P Boffetta, et al. European Journal of Epidemiology. Under Revision.

and three non-European countries. During the first four years of the project (2010-2013), a total of 43 research proposals, have been developed. Different research hypotheses are currently investigated with meta-analyses and collaborative papers have already appeared in the literature[1]. CHANCES results may help international organizations, governments and policy-makers to better understand the consequences of ageing and, thus, make informed decisions.

Acknowledgements: CHANCES has received funding from the European Union Seventh Framework Programme under grant agreement HEALTH–F3-2010-242244.

1 B Schöttker B, et al. BMJ 2014; 348:g3656.

SESSION 2
Stem cells, regenerative medicine and aging

INVITED SPEAKERS

Cellular senescence in tissue remodelling: from physiology to pathology

Muñoz-Espín. D[1], Serrano. M[1]

Recent discoveries are re-defining our view of cellular senescence as a trigger of tissue remodeling that acts during normal embryonic development and upon tissue damage. In the case of developmental processes, cellular senescence facilitates the elimination of transient structures, controls the balance of different cell populations, and contributes to the regulation of morphogenesis. To achieve tissue renewal, senescent cells arrest their own proliferation, recruit phagocytic immune cells, and promote the mobilization of nearby progenitor cells that repopulate the tissue. This sequence of events (senescence, followed by clearance and then regeneration) may not be efficiently completed in aged or pathological contexts, thereby resulting in the accumulation of senescent cells that aggravates tissue dysfunction. Increasing evidence indicates that manipulation of cellular senescence can be used as an innovative therapeutic tool. In cancer and during active tissue repair, pro-senescent therapies contribute to minimize the damage by limiting proliferation and fibrosis, respectively. At the same time, anti-senescent therapies may help to eliminate senescent areas that accumulate during ageing or chronic damage and recover tissue function.

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Human adipose-derived stem/progenitor cell aging and caloric restriction

Zwerschke. W[1]

Aging is associated with a decline of physiological functions of tissues, organs and the whole organism that has origins in cellular deterioration. Long-term caloric restriction (CR) without malnutrition induces health and maximum life span extension in a wide variety of species. A better understanding of the effects of CR on specific cell types and the respective tissues will lead to new insights into the aging process. In humans, long-term CR is a key effect of prolonged reducing diets and bariatric surgery procedures. We study the impact of these interventions on adipose-derived stromal/progenitor cells (ASCs), which are crucial for adipose tissue homeostasis. In my presentation I would like to speak about the aging phenotype of ASCs isolated from abdominal subcutaneous fat pads and reprogramming of these cells by long-term CR in formerly obese humans.

Begin at the beginning and go on till you come to the end; then stop

Yanai. H[2], Taranukha. D[2], Vierlinger. K[3], Hofner. M[3], Nöhammer. C[3], Chilosi. M[4], Budovsky. A[2], **Fraifeld. VE**[2]

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Does the longevity phenotype have any advantage in the ability to repair damaged tissues? In an attempt to answer this question, we explored skin wound healing (WH) in the long-lived transgenic αMUPA mice, a unique model of genetically extended life span. These mice spontaneously eat less, preserve their body mass, are more resistant to spontaneous and induced tumorigenesis and live longer, thus greatly mimicking the effects of caloric restriction (CR). We found that αMUPA mice showed a much slower age-related decline in the rate of WH than their wild-type counterparts (FVB/N). After full closure of the wound, gene expression in the skin of old αMUPA mice returned close to the basal level. In contrast, old FVB/N mice still exhibited significant upregulation of genes associated with growth-promoting pathways, indicating an ongoing tissue remodeling or an inability to properly shut down the repair process. It appears that the CR-like longevity phenotype is associated with more balanced and efficient WH mechanisms in old age, which could ensure a long-term survival advantage. In essence, it is attractive to suggest that attenuating the age-related decline in feedback responses could be one of the keystones of longevity-promoting interventions. It seems that Lewis Carroll was quite right when he wrote: "Begin at the beginning and go on till you come to the end; then stop".

POSTERS

Is there a role for accelerated fibroblast senescence and p38 MAPK activation in human progeroid syndromes?

Davis. T[1], Kipling. D

This poster provides a summary of our work over the last few years on fibroblast senescence in progeroid syndromes. Human ageing studies are problematic due to their complex nature. Genetic progeroid syndromes that manifest a subset of ageing phenotypes are powerful proxies to dissect out how specific mechanims of ageing, such as cellular senescence, may

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play a role in the overall ageing process. Cellular senescence occurs as a result of telomere dysfunction (replicative senescence) or via activation of p38 MAP kinase (stress-induced senescence), with senescent cells gradually building up during life. For several progeroid syndromes, notably Werner, ATR-Seckel, Hutchinson-Gilford, Ataxia-Telangiectasia, Nijmegen Breakage and Dyskeratosis Congenita, there is clear evidence that fibroblasts undergo rapid or premature cell ageing. Other syndromes such as Cockayne, Rothmund-Thomson and Bloom show no such clear evidence. In some instances no clear relationship between the severity of ageing features and premature fibroblast senescence is seen, although as some of these syndromes result in early death from non-age related causes, it may be that insufficient lifespan is available for significant *in vivo* premature ageing to occur. An additional complexity is the possibility that there is tissue specificity with regard to where premature cell senescence occurs in each syndrome, which further complicates interpretation. Nevertheless, a coherent story is starting to emerge whereby accelerated fibroblast ageing that does occur results from accelerated telomere dysfunction in some syndromes, activation of p38 in others, or a mix of both mechanisms.

SESSION 3
Metabolic regulation in aging

INVITED SPEAKERS

The Influence of Dietary Fat Source on Mitochondria and Life Span in Calorie Restricted Mice

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A number of different diets have been used to investigate the impact of calorie restriction (CR) on life span. However, it is not known if there is an optimum diet composition to maximize life span extension with CR. The purpose of the present study was to determine the influence of dietary fatty acid composition on mitochondrial membrane composition, mitochondrial function and life span. Male C57BL/6J mice were divided into four groups (control and 3 CR groups) and fed AIN93M diets at 95% (control) or 60% of ad libitum intake. The primary dietary fats for the three CR groups were soybean oil (high in n-6 fatty acids), fish oil (high in n-3 fatty acids) or lard (high in saturated and monounsaturated fatty acids). The C group was also fed the soybean oil diet. Mitochondrial phospholipid fatty acid composition was altered in a manner which reflected the unsaturated fatty acid profiles of the diets. The CR lard group had a decreased ($p < 0.05$) peroxidizability index compared to CR fish for skeletal muscle and both CR fish and CR soy for liver mitochondrial phospholipids. Dietary fat source also altered proton leak, H_2O_2 production, and electron transport chain enzyme activities in a tissue-dependent manner. Life span was increased ($p < 0.05$) in the CR lard

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group compared to both the CR fish and CR soy groups. The results of this study suggest that diets containing a high proportion of monounsaturated and/or saturated fatty acids may maximize life span extension with CR.

Towards a unifying biochemical mechanism of metabolism, longevity and aging

Sollott. SJ[1]

A growing list of interventions beyond dietary restriction has been shown to prolong lifespan and modify healthspan across a variety of experimental animal systems. Although a variety of distal-effector mechanisms have been examined (e.g., involving mitochondrial function, autophagy, telomeres, p53), a unifying biochemical process that controls these has yet to be proven. We have found that controlling the citrate/acetyl-CoA flux in the cell achieves regulation of key protein acylation/de-acylation reactions, which in turn modify the functions of integral proteins and enzymes involved in directing metabolism, biogenesis, aging, and longevity.

Adipose tissue and ageing: let's get the fats straight

Brown. J[2]

Increases in longevity and the incidence of obesity and metabolic diseases such as diabetes present significant challenges to all societies in the 21st

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century. Despite increased longevity, decreases in the relative amount of time spent in good health, so called 'healthspan', are becoming apparent. This is thought to be in part due to the increased prevalence of metabolic disease. It is established that as we age our metabolic tissues lose some of their normal function, with changes in insulin sensitivity and nutrient homeostasis being common place. This is due not only to direct age-related decline of metabolic tissues, but also potentially from the influence of senescent cells, which we have shown can influence glucose and lipid homeostasis in metabolic cells. A growing amount of evidence also supports now supports the notion that the relationship between metabolic tissues and ageing is a two way street, with a potential role in regulation of cellular ageing for hormones released from adipose tissue (adipokines such as leptin and adiponectin) and muscle (myokines such as irisin). This suggests that our body composition can impact upon how well we age and our 'healthspan'. Potentially key in this is irisin, a myokine released post-exercise which we have reported is positively associated with telomere length. Evidence for the role of adipokines and myokines in the ageing process are discussed.

ORAL COMMUNICATIONS

Dietary fat source modulates metabolic gene expression in calorie restricted mice

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Calorie restriction (CR) extends life span and delays the onset of several age-related diseases. CR mice for which saturated (SFA) and monounsaturated fatty acids (MUFA) are the primary dietary fat source exhibit increased longevity compared to those fed diets containing polyunsaturated fatty acids (PUFA, both n-3 and n-6). Since PUFA are known to be active regulators of gene expression, we have used deep sequencing techniques (RNA-sequencing and advanced pathway analysis) to explore possible mechanisms underlying the observed life span effect.

Extensive transcriptional regulation is found in the liver, whereas skeletal muscle gene expression appears to be less sensitive to regulation by CR or dietary fat. Several transcription factors, cytokines and signal transducers could mediate calorie restriction effects according to our results. Gene expression through PPARα, PPARγ and related transcription regulators, as well as TNF signaling, is inhibited by CR in liver.

Dietary fish oil, but not soybean oil, activates PPARα signaling, and several other transcription regulators. SREBFs are activated in lard-consuming animals compared to diets containing either fish or soybean oil. The expression of several rate-limiting enzymes and key regulators of metabolism is also altered by dietary fatty acids.

This study shows that dietary fatty acids modulate gene expression in calorie restricted mice, and identifies the most relevant transcriptional regulators. We have also detected changes in regulators and effectors of energy metabolism, which are sensitive to dietary fatty acids and CR and may contribute to life span extension in CR mice.

Site-specific ROS signal preserves mitochondrial function and extends Drosophila lifespan

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Increased reactive oxygen species (ROS) production has long been considered a deleterious hallmark of aging and many degenerative diseases. However, several recent studies have implicated ROS as vital secondary messenger molecules in cell differentiation and stress adaptation. Here we show that site-specificity of ROS signalling could explain the apparent dual nature of ROS. We report that ROS increase in parallel to the decrease in mitochondrial respiration during ageing in *Drosophila melanogaster*. Knockdown of mitochondrial superoxide dismutase increases ROS levels and dramatically shortens lifespan. Paradoxically, it is possible to rescue this phenotype increasing mitochondrial ROS production via expression of an alternative NADH dehydrogenase internal 1 (NDI1). NDI1 over-reduces the mitochondrial ubiquinone pool (CoQ) generating a mitochondrial H_2O_2 signal that activates mechanisms of quality control and extends lifespan in *sod2* mutants and wild type flies. When the mtH2O2 signal is supressed through expression of an alternative oxidase (AOX) the lifespan extension is also supressed. Interestingly, mutations in *pink1* block reduction of CoQ causing a similar phenotype than mutations in *sod2*. Once again, we rescued *pink1* mutations restoring reduction of CoQ and increasing ROS levels via expression of NDI1. In summary, we show the existence of a mitochondrial H2O2 signal that is produced as a consequence of the over-reduction of the

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CoQ. Increasing this signal is possible to rescue mutations in *pink1* and increase lifespan in wild type flies, indicating that manipulation of CoQ redox state is a valid strategy to delay ageing and age-related diseases.

POSTERS

Alterations of mitochondrial physiology and mTOR pathway makers by glucose levels and exogenous lipids in an in vitro model

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Unsaturation degree of membrane phospholipids (Membrane Theory of Aging) (*J ExpBiol* .2003. *206:2303*) and endogenous generation of reactive oxygen species (ROS) (Mitochondrial/Free Radicals Theory of Aging) (*J Gerontol* .1956. 11: 298) are two of the most widely accepted factors that affect aging. Dietary fatty acids can modify the composition of membrane phospholipids (*J NutrBiochem*. 2001. 12: 357), and the function of the mitochondrial electron transport chain (ETC) (*Br J Nutr* .2012**.** *107: 647*).The Ser/Thr-kinase mTOR is crucial in the sensing of nutrient and growth factors availability, and in regulating autophagy. Optimal nutrient sensing through the mTOR pathway is impaired with aging and reversely, inhibition of mTOR pathway increases lifespan (*Cell*. 2013.6; 153:1194).Our aim was to determine how mitochondrial physiology, cellular oxidative damage and mTOR signaling pathway are affected by supplementation with exogenous lipids in an *in vitro* model. Mouse hepatocarcinoma Hepa 1.6 cells were grown in medium containing either low (1g/l) or high glucose (4,5g/l), and supplemented or not with two lipid emulsions at 7µl/ml: *Lipofundin*

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(ω-6 fatty acids) or *Lipoplus* (also containing ω-3 fatty acids). After 48 hours of treatment, different parameters related with mitochondrial function (including mitochondrial potential and activities of ETC complexes) and oxidative stress (including superoxide and peroxide levels and oxidative damage), as well as the activation state of mTOR pathway components were assessed.Our results indicate that exogenous lipids target mitochondrial physiology, oxidative stress and mTOR pathway in a manner that is dependent on fatty acid composition.

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Effects of aerobic interval exercise combined with resistance training versus caloric restriction on metabolic and inflammatory markers of obese rats

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Introduction: The most frequently used methods to struggle against obesity and metabolic syndrome are caloric restriction (CR) and exercise[1-2]. High-intensity exercise may be more beneficial than moderate-intensity exercise at improving metabolic syndrome markers[3]. Resistance training has also important metabolic effects such as reduced fat mass and plasma LDL-cholesterol and triglycerides[4]. We aimed to compare the metabolic effects of a training program based on aerobic interval exercise combined with resistance training (AIEaRT) with CR in obese rats. **Methods:** Thirty-two obese Zucker rats were randomly divided into 4 groups: sedentary or exercise with or without CR. The training groups conducted an AIEaRT

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program in the same 60 min session, 5 days/week for 2 months. Body weight, fat and muscle mass, plasma fasting glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, phospholipids and triglycerides, as well as tumour necrosis factor alpha (*TNF*-α) and interleukin (IL) 1and IL-10 were measured. **Results:** Final body weight was lower in the CR groups ($p<0.001$). Fat mass was lower in the CR ($p<0.05$) and AIEaRT groups ($p<0.001$), while the AIEaRT also positively increased lean mass ($p<0.001$). Plasma triglycerides were lower in the CR group ($p<0.001$) whereas plasma total and LDL-cholesterol and fasting glucose were reduced only in the AIEaRT groups ($p<0.001$). Plasma phospholipids decreased in CR groups ($p<0.05$) and especially in the AIEaRT groups ($p<0.001$). Finally, despite CR as well as AIEaRT both seem to decrease plasma cytokines, the differences were not significant. **Conclusions:** Overall, AIEaRT showed greater improvements on body composition, lipid profile and glycaemia than CR, without a clear positive interaction between both interventions.

Keywords: metabolic syndrome, interleukin, tumour necrosis factor alpha, body composition, cholesterol, phospholipids, triglycerides.

SESSION 4
Antioxidant systems in aging

INVITED SPEAKERS

Cytochrome B5 reductase 3 over-expression extends lifespan in mice

Bernier. M[1], Martin-Montalvo. A[1], Gutiérrez. V[1], Palacios. HH[1], Navas. P[2], Villalba. JM[3,] de Cabo. R[1]

Understanding the mechanisms by which metabolism is controlled is critical in order to achieve therapeutic strategies for the treatment of metabolic diseases and aging. Cytochrome B5 reductase 3 (CYB5R3) is required for the elongation and desaturation of fatty acids, cholesterol synthesis and mono-oxygenation of cytochrome P450 enzymes, all of which are associated with protection against metabolic disorders. We generated transgenic mice over-expressing CYB5R3 to investigate the function of this enzyme in mouse physiology. When overexpressed, CYB5R3 improved several metabolic parameters resulting in lifespan extension (7.1% increase in maximal lifespan). We determined that CYB5R3 over-expression partially protects from d*iethylnitrosamine-induced* hepatocarcinoma. *CYB5R3* transgenic mice accumulated high levels of long chain poly-unsaturated fatty acids in association with an overall improvement in mitochondrial function, decreased oxidative damage accumulation and inhibition of chronic pro-inflammatory pathways. Our findings indicate that CYB5R3 represents a new target in the study of genes that control lifespan.

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Old dog with new tricks; role of NQO1in metabolic regulation

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Understanding basic molecular and physiological mechanisms that govern metabolic homeostasis is critical to develop aging and age-related metabolic interventions. By virtue of its ability to lower oxidative stress and adjust the levels of intracellular NAD+, the plasma membrane redox system (PMRS) constitutes a key factor in the control of these processes. Long-term caloric restriction without malnutrition has been shown to increase the activity and levels of a known member of the PMRS system, NAD (P) H: Quinone Oxidoreductase 1 (NQO1). NQO1 catalyzes the two-electron reduction of quinones using NADH or NADPH as electron donors, thus contributing to the detoxification of chemicals and ROS. In addition, NQO1 has been associated with protection against mutagenesis and carcinogenesis. Herein, we have generated a transgenic mouse strain that constitutively over-expresses the rat NQO1 gene to investigate the effects of NQO1 in the context of aging and metabolic diseases. Our results indicate that the beneficial effects of CR on longevity are not solely related to NQO1, because the survival curve, as well as body weight, body composition, food intake, and energy balance of NQO1-Tg mice was similar to Wt mice when maintained in standard diet. However, under metabolic stress conditions, over-expression of NQO1 improves healthspan in mice, as NQO1-Tg mice fed a high-fat diet showed lower body weight and improvement in physical performance

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than their Wt counterparts. Interestingly, NQO1-Tg mice exhibited lower chronic inflammation and liver integrity was protected in NQO1-Tg obese mice. Moreover, over-expression of NQO1 enhanced insulin sensitivity, as indicated by lower levels of fasting glucose, serum insulin, and HOMA-IR. Taken together, these data clearly illustrates that NQO1 has a role in the maintenance of glucose homeostasis, and pharmacological interventions targeting NQO1 may constitute a therapeutic approach to control metabolic and age-related metabolic diseases.

ORAL COMMUNICATIONS

Effects of antioxidant supplementation in different age groups

Jansen. E[1]

Introduction. The specific aim for this project is to test the hypothesis that supplementation of antioxidant vitamins in humans have positive health effects only in later stages of adult life. This biomarker driven project consists of two parts, a short-term human intervention study and a lifetime mice study both with a low-dose of multi-vitamins/minerals. In the present paper an overview will be given of both studies with focus on the oxidative stress, redox and antioxidant status.

Methods. The human intervention study was organized as a randomized intervention trial, included 80 adult, stratified by sex and age. The participants received a multivitamin/mineral preparation containing a low dose during 8 weeks. Serum, plasma and erythrocytes were sampled at three time points.

The lifetime mice study (600 mice) was performed with feed containing two different dose levels of multivitamin/minerals.

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Results. In the human study, biomarkers of some vitamins increased significantly. The total thiols as a serum biomarker of redox status, however, showed an aging-related decrease after 8 weeks. In erythrocytes, the antioxidant enzymes of the glutathione pathway increased significantly whereas total glutathione did not change. The results from the mice study show a chance in the glutathione synthesis.

Conclusion. Supplementation of human volunteers with a low-level of multivitamins and minerals resulted in a change of the redox status both in serum (age-related) and erythrocytes. The lifetime mice study showed also disturbances in the glutathione pathway.

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Reductive stress in young healthy individuals at risk of Alzheimer´s disease

Lloret. A[1], Badia. MC[1], Giraldo. E[1], Fuchsberger. T[1] and Vina. J[1]

Apolipoprotein E4 (ApoE4) is a major genetic risk factor for the development of Alzheimer's disease (AD). Oxidative stress is a hallmark of AD but this has not been studied in young healthy persons at risk of the disease. The aim of this work was to find if carrying ApoE4 alleles correlates with oxidative stress markers and with molecular changes associated with specific processes involved in AD pathophysiology. However, our results showed that lymphocytes from young, healthy persons carrying at least one Apoε4 allele suffer from reductive rather, than oxidative stress, i.e., lower oxidized glutathione and P-p38 levels and higher expression of enzymes involved in antioxidant defence like glutamyl cysteinyl ligase and glutathione peroxidase. In contrast, in the full-blown disease, the situation is reversed and oxidative stress occurs, probably due to the exhaustion

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of the antioxidant mechanisms just mentioned. Moreover, young healthy persons carrying the ApoE4 genotype express more RNA for RCAN1, calcineurin, and PKR, GSK3β and phospho-tau than controls (ApoE 3/3). Moreover, we found that carrying one or two alleles for ApoE4 is associated with subjective cognitive impairment. We conclude that young, non-demented persons carrying the ApoE 4/4 genotype show reductive stress and molecular changes that are involved in specific processes associated with the pathophysiology of AD such as increased phosphorylation of tau or increased expression of stress-related proteins like calcineurin, GSK3β, or RCAN1. These changes may help to understand the development of AD and in the early diagnosis of the disease.

POSTERS

The resveratrol effect on human T-lymphocytes Sirtuin 1 modulation is influenced by a Superoxide Dismutase 2 gene polymorphism

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Aging process can causes a chronic production of cytokines leading to an unresolved inflammatory response. This process is intensifying by oxidative stress. Polyphenol molecules, as resveratrol can act on peripheral blood mononuclear cells (PBMCs) decreasing their proliferation and inflammatory cytokines, by upregulation of some anti-aging genes, as Sirtuin 1. However, if this property is influenced by PBMC basal oxidative state is an open question. Therefore, we evaluated the resveratrol effect on human PBMC

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activation carriers different genotypes of a single nucleotide polymorphism (SNP) found in manganese superoxide dismutase (SOD2) gene. This SNP located in codon 16, (rs4880) encodes for either alanine (Ala) or valine (Val) amino acids. AA and VV homozygous genotypes are associated with risk to some cancer and cardiometabolic diseases, respectively. Initially, 120 healthy adult volunteers were genotyped to Ala16Val-SOD2 SNP by tetra-primer ARMS-PCR analysis (genotype frequencies: AA=26.7%; VV= 28.2%; and AV=45.1%). Blood sample was again obtained from a sub-sample and PBMCs activated were concomitantly exposed to different resveratrol concentrations (2, 5, 10 and 30 μM). The resveratrol effects were evaluated on PBMCs proliferation (MTT assay), cytokines (IL- β, IL-6, TNFα, Igγ and IL-10) and Sirtuin 1 levels (ELISA immunoassays). Sirtuin 1 gene expression was also determined (qT-PCR analysis). The proliferation decreased in heterozygous cells (AV) exposed to resveratrol (≥ 2μM), as well as inflammatory cytokines. At contrary, VV-PBMCs increased cell proliferation when exposed to resveratrol. However, Sirtuin 1 gene was upregulated by resveratrol independent of Ala16Val-SOD2 genotypes. The results suggest that resveratrol antiinflammatory effect is not universal.

Are autophagy and mitophagy novel players in UVB-induced senescence of human dermal fibroblasts?

Cavinato Nascimento. MA[1], Koziel. R,[1], Hermann. M[2], Jansen-Dürr. P[1]

Skin aging is a progressive multifactorial degenerative process, that results from two synergic mechanisms: intrinsic (chronological) and extrinsic (photoaging)[3]. It is known that repeated exposure to mild doses of UVB

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3 Fisher GJ, Kang S, Varani J, et al. Mechanisms of photoaging and chronological skin aging. Arch Dermatol. 2002; 138(11):1462-1470. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12437452

light triggers stress-induced premature senescence of Human Dermal Fibroblasts (HDFs)[1]. UV and other forms of ionizing radiation cause skin injury through the generation of free radicals, reactive aldehydes, oxidative protein as well as DNA damage. This molecular damage can be removed by the lysosomal system, in a process called autophagy, and the alterations in this pathway lead to accumulation of damaged molecules. Autophagy can also modulate cell viability under stress conditions and recent evidence links autophagy to cellular senescence[2]. Exposure to UV radiation results in accumulation of damage to the mitochondrial DNA of dermal fibroblasts leading to altered gene expression which chronically drives the aging process. Damaged mitochondria are removed in the process called mitophagy[3]. Using the model of UVB-induced senescence we adressed the effect of UVB light on the mitochondrial and lysosomal networks and other physiological parameters.

Biomarkers of oxidative stress in aging studies

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Introduction.Oxidative stress has been proposed to be important in age-related processes and chronic diseases. In the EC-FP7 project CHANCES

1 Greussing R, Hackl M, Charoentong P, et al. Identification of microRNA-mRNA functional interactions in UVB-induced senescence of human diploid fibroblasts. BMC Genomics. 2013; 14(1):224. Doi: 10.1186/1471-2164-14-224.

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a biomarker approach was used to study both the aging process and the prevalence of chronic diseases.

Methods. Biomarkers were measured in serum samples of the 8-year follow-up of the ESTHER study (2,932 participants from Germany) and of the HAPIEE study (5,379 participants from a multi-center cohort studyin Krakow, Kaunas and Prague). As biomarker of oxidative stress, the assay for Reactive Oxygen Metabolites (ROM) was chosen, the Biological Antioxidant Potential (BAP) for anti-oxidant status and the Total Thiol Levels (TTL) for the redox status. The assays were adapted for use on an auto-analyzer.

Results. The three biomarkers were tested for their performance and stability on short- and long-term storage. They were applied for the first time inlarge-scale studies. ROM (positive) and TTL (negative) had statistically significant associations with all-cause mortality in the ESTHER study, and with total CVD mortality in the HAPIEE study. Furthermore, in the ESTHER study, a statistically significant positive association with frailty was observed for ROM and an inverse association for TTL.

Conclusion. This is the first large scale study where the three biomarkers ROM, BAP and TTL were used. The results suggest that ROM and TTL are risk markers for CVD mortality, all-cause mortality and frailty.

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Life span impairment of Nrf2 MEFs lacking the antioxidant response transcription factor Nrf2: Role of apoptotic cell death

Ariza. J[1], Jódar. L[1], González-Reyes. JA[1], de Cabo. R[2], Villalba. JM[1]

Nuclear factor E2-related factor-2 (Nrf2) **(Moi et al., 1994)** is a transcription factor serving as cellular sensor of oxidative and electrophilic stress generated from endogenous reactions and exogenous chemicals, xenobiotics, drugs, UV, and ionizing radiations **(Kwak et al., 2003; Jaiswal et al., 2004; Lee et al., 2005)**. Upon its activation and stabilization, Nrf2 rapid translocates to the nucleus where it activates its target genes by binding to the antioxidant response element (ARE) **(Jain and Jaiswal, 2005)**, a common regulatory element of genes coding for antioxidant and detoxification enzymes. Although Nrf2 plays a key role in preventing from aging-related carcinogenesis in mice under calorie restriction, it is dispensable for to the pro-longevity effects **(Montalvo et al., 2011)**. However, a decline in levels of Nrf2 in aged organisms that promotes oxidative damage is well documented **(Suh et al., 2004).** Also, increased Nrf2 activity was linked with extended life span in cultured human fibroblasts **(Lerner et al., 2013)** while Nrf2 silencing led to a premature senesce **(Kapeta et al., 2010).** In order to assess the effects of Nrf2 deletion on *in vitro* cellular life span we used immortalized wild type and Nrf2KO MEFs. We found out that genetic deletion of Nrf2 diminished growth rate and shortened lifespan. Several apoptotic markers were significantly increased in Nrf2KO cells in comparison to their Wt counterparts. In addition, Nrf2 deletion impaired proliferation markers and produced mitochondrial alterations. Decreased

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proliferation, increased apoptotic signaling and mitochondrial dysfunction may be related with a decrease of life span in Nrf2KO cells.

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Exchange of GSH across human muscle. Role of exercise and age

Cabo. H[1], Salvador-Pascual. A, Sabater-Pastor. F, Ferrando. B, Pareja-Galeano. H, Gómez-Cabrera. MC, Vina. J

Reactive oxygen species (ROS) can be deleterious to cells when not sufficiently counterbalanced by the antioxidant system. Aging is associated with accumulation of oxidative damage to macromolecules. Therefore, skeletal muscle formation of ROS in response to exercise could be excessive, which potentially causes cellular damage in the aged state. The purpose of the present study was to examine the effect of acute exercise on exchange of GSH and GSSG across the leg in young and older sedentary and older lifelong physically active humans.

We evaluated the effect of acute exercise on changes in blood redox state across the leg of young (23±1 years) and older (66±2 years) sedentary humans by measuring the whole blood concentration of GSH and GSSG. To assess the role of physical activity, lifelong physically active older subjects (62±2 years) were included.

Exercise increased the venous concentration of GSSG in an intensity-dependent manner in young sedentary subjects, suggesting an exercise-induced increase in ROS formation. In contrast, venous GSSG levels remained unaltered during exercise in the older sedentary and active groups despite a higher skeletal muscle expression of the superoxide generating enzyme NADPH oxidase. Arterial concentration of GSH and expression of

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antioxidant enzymes in skeletal muscle of older active subjects was found to be increased. The potential impairment in exercise-induced ROS formation may be an important mechanism underlying skeletal muscle and vascular dysfunction with sedentary aging.

Lifelong physical activity up-regulates antioxidant systems which may be one of the mechanisms underlying the lack of exercise-induced increase in GSSG. Doi: 10.1016/j.freeradbiomed.2014.05.008

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High-intensity exercise modifies the effects of anabolic androgenic steroids on brain oxidative stress in rats

Camiletti-Moirón. D[1], Aparicio. VA[1], Nebot. E[1], Medina. G[1], Martínez. R[1], Kapravelou. G[1], Andrade. A[1], Bernier. M[2], de Cabo. R[2], Porres. JM[1], López-Jurado. M[1] and Aranda. P[1]

Anabolic androgenic steroids (AAS) may promote neurodegenerative and apoptotic effects[3], while regular exercise plays a preventive role in reducing these adverse events[4]. However, the benefits of high-intensity

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exercise (HIE) on brain redox status are under debate[1]. Here, we analysed the effects of HIE and AAS on brain redox status. **Methods:** Forty male Wistar rats were randomly distributed in 4 experimental groups (n=10) with or without HIE, usually employed by HIE practitioners, and with or without weekly administration of AAS (10mg/kg body weight). Thiobarbituric acid-reactive substances (TBARs) and protein carbonyl content (PCC) were assessed. Total superoxide dismutase (tSOD), manganese superoxide dismutase (Mn-SOD), cooper/zinc superoxide dismutase (CuZn-SOD) and catalase (CAT) activities were measured. Finally, protein expression level of glutathione peroxidase (GPx), NAD(P)H dehydrogenase, Quinone 1 (NQO1), NF-E2-Related Factor 2 (Nrf2), glial fibrillary acidic protein (GFAP), nuclear factor kappa B p65 (NF-κB) and signal transducer and activator of transcription 3 (STAT3) were determined. **Results:** Brain PCC concentrations were lower in the HIE groups compared to the sedentary controls, whereas CAT activity was higher (both, $p<0.01$). Both HIE and AAS groups exhibited higher expression of GFAP and GPx, but lower NQO1 levels (all, $p<0.05$). There was increased expression of NF-κB in the AAS groups ($p<0.01$). Several HIE*AAS interactions were found on TBARs content and NF-κB expression, with HIE downregulating AAS-mediated increase in NF-κB ($p<0.05$). **Conclusions:** Overall, HIE appeared to reduce the AAS-mediated negative effect on brain redox status.

Mitochondrial oxidative stress in aging. Is it coenzyme Q-dependent?

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1 Camiletti-Moirón D, Aparicio VA, Aranda P, Radak Z. Does exercise reduce brain oxidative stress? A systematic review. Scand J Med Sci Sports 2013; 23:e202-12.

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Both oxidative stress and coenzyme Q play a key role in ageing process. We studied the relationship among oxidative stress, ageing and coenzyme Q levels in mice liver. A subcellular fractionation protocol has been optimized and adapted to mouse cells. Oxidative stress has been measured in mitochondrial fraction by determining levels of peroxidation and protein nytrotyrosine levels, in both cases increasing levels were found in mitochondria from aged animals (18 months) in comparison with young (1 month) or mature (6 months) animals. This higher oxidative profile was accompanied by a decrease in the glutathione peroxidase activity and an increase in cytochrome B5 reductase activity. Levels of coenzyme Q also decreased in 18 months livers although affecting to Q9 whereas Q10 levels increased, probably indicating a higher need to protect against oxidative damage. This effect produced a clear decrease in the ratio Q9/Q10 in liver mitochondria with age. This tendency was accompanied by a lower level of CoQ7 protein in mitochondria. In general, these preliminary results seem to demonstrate that coenzyme Q can play an important role in protection against oxidative damage in mitochondria during aging.

CYB5R3 at the intersection of growth and senescence

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Metabolic events regulating cell growth and survival can lead organisms towards health or disease depending on the context. For example, after serum withdrawal, cells survive thanks to the activation of the fork-head transcription factor FOXO3a, through the regulation of detoxification genes, a direct consequence of the lack of AKT activity in absence of growth factors. We shows evidences that FOXO3a regulates the expression of mammalian membrane-bound NADH-cytochrome b5 reductase (*CYB5R3*), an essential enzyme in humans and the deficiency of its activity in non-erythroid cells

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by mutations in its gene results in neurological disorders associated to type II methemoglobinemia. Lacking of CYB5R3 activity induces senescence and its expression is reduced in old and senescent cells. Thus, mammal *CYB5R3* would be at the crossroads between growth and senescence.

These data are partially been reported in ANTIOXIDANTS & REDOX SIGNALING Volume 21, Number 12, 2014. pp: 1708-1725.

SESSION 5
Neuro/muscle in aging

INVITED SPEAKERS

Aging at different speeds: rodent in vivo model

Gruart. A[1], López-Ramos. JC[1], Muñoz. MD[2], Delgado-García. JM[1]

A definitive consequence of aging is the progressive deterioration of higher cognition functions as well as motor capabilities. Different studies developed in our laboratory using rodent models showed that aging does not follow a unique trend across time, but it can be accelerated or decelerated. It is claimed that one of the main contributions to this acceleration in the aging speed is the presence of neuronal diseases, such as Alzheimer disease. We determined the learning capabilities of 3-, 12-, and 18-month-old wild-type and single-transgenic (APP751SL, PS1M146L) and double-transgenic (APP751SL /PS1M146L) mice using the classical conditioning of eyelid responses. Results showed that the main learning and memory deficits observed in aged wild-type and transgenic mice were not directly related to the genetic manipulations or to the presence of amyloid plaques, but they showed a close correlation with animals age. We have also tested the aging deceleration after physical exercise or genetic manipulations. Physical exercise improved the sensorimotor function and the brain tissue antioxidant defense in both 3xTg-AD and NonTg mice. The benefits of aerobic physical exercise on synapse, redox homeostasis, and general brain functions demonstrated —in 3xTg-AD mice— the relevance of a healthy life-style. Surprisingly, we also demonstrated that defects in DNA repair mechanisms (Polμ-/-) maintain the ability to learn at ages when wild-type mice do not. This is the first example in which the genetic ablation of a DNA-repair function results in a substantially better maintenance of learning abilities, together with fewer signs of brain aging, in aged mice.

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Influence of age on leptin induced skeletal muscle signaling

Guadalupe-Grau. A[1, 2]

Leptin is a 16 KDa hormone primarily released from adipocytes and the systemic levels increase in direct proportion with increasing adiposity. Leptin has been highlighted as a promising anti-obesity therapeutic target because of its central role in food intake and body weight control. Pleiotropic effects have also been attributed to leptin due to the expression of leptin receptors (OB-Rs) in several peripheral tissues, such as skeletal muscle. In addition to its locomotive function, skeletal muscle accounts for the majority of the basal metabolic rate and is the primary tissue responsible for whole body glucose metabolism. Leptin stimulates fatty acid oxidation, reduces the accumulation of intramuscular fat and increases glucose uptake and energy expenditure in skeletal muscle, but the intracellular mechanisms leading to these effects are not completely understood. The aging process is associated with lower qualitative mitochondrial respiratory capacity (Larsen et al., 2012), increased ectopic fat mass and plasma leptin levels (Carter et al., 2013) concomitant with a progressive reduction of systemic androgen availability (i.e. free testosterone) (Wang et al., 2009). Therefore, the aim of this talk will be to address the research on the effects of ageing on the amount of leptin receptors as well as its intracellular pathways in human skeletal muscle. We will focus on mechanisms leading to intramuscular leptin resistance, as well as on interventions that induce activation of leptin signaling in human skeletal muscle, like exercise.

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ORAL COMMUNICATIONS

Learning capabilities and CA1-prefrontal synaptic plasticity in animal models of accelerated or pathological senescence. Protective effects of T588 and physical exercise

López-Ramos. JC[1]

The study of learning and memory deficits that are pathognomonic of the normal or pathological aging processes requires the availability of suitable animal models. In this regard, senescence-accelerated-prone 8 (SAMP8) mice represent a suitable model of accelerated senescence as compared with SAMR1 animals presenting normal aging. On the other hand, the Alzheimer Disease triple transgenic mouse model (3xTgAD) develops a partially similar neuropathology to Alzheimer's disease, especially severe from 12 months of age. In several studies, we applied electrophysiological and behavioral techniques to characterize learning capabilities and synaptic changes in those animals. For that, intracerebral stimulatory electrodes were implanted in the hippocampal CA1 area, and recording ones in the medial prefrontal cortex. Moreover, we evaluated the protective effects of the drug T588, and the practice of voluntary exercise in a running wheel.

Results showed infra-activity of the CA1-prefrontal pathway in SAMP8 mice, in comparison to SAMR1 ones, and over-activity of the CA1-prefrontal pathway in 3xTg-AD animals, in comparison to the NoTg ones, but the induction of Long Term Potentiation (LTP) had only potentiating effects in SAMR1 and in NoTg mice. Behavioral tasks demonstrated certain neuroprotective effects of T588 or physical exercise in SAMP8 and 3xTg-AD mice, respectively.

In conclusion, these studies showed the different patterns of synaptic dysfunction in normal and pathological aging, and the partially protective effects of T588 or voluntary exercise.

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POSTERS

Physical exercise partially revert synaptic plasticity deficits in 3xTg-AD mice

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3xTgAD mice develops a partially similar neuropathology to Alzheimer disease, with β-amyloid deposits, hyperphosphorilated tau and altered neurotransmission systems, among other alterations, especially severe from 12 months of age. Thus, 3xTgAD mice represent a good model to study pathologic aging and neuroprotective therapies. Physical exercise has neuroprotective effects, as the slowdown of cognitive impairments in mature and aging humans. In this study, we applied electrophysiological techniques to characterize synaptic changes in animals housed in standard conditions, or with free access to an exercise wheel. For that, intracerebral stimulatory electrodes were implanted in the hippocampal CA1 area, and recording ones in the medial prefrontal cortex. We used male 3xTg-AD mice of 7 months of age (which had been making volunteer exercise during 6 months), sedentary 3xTG-AD mice, and sedentary control NoTg mice. Animals also performed selected behavioral tasks, as pre-pulse inhibition of the startle response, and operant conditioning in Skinner box.

Results showed over-activity of the CA1-prefrontal pathway in 3xTg-AD animals, in comparison to the NoTg ones, correlating with a stronger startle response and impaired pre-pulse inhibition. Nevertheless, experimental induction of Long Term Potentiation (LTP) in CA1 only had potentiating effects in NoTg animals, but not in 3Tg-AD ones. In addition, the operant conditioning task showed an improvement of the 3xTg-AD exercise group

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along the sessions, reaching values similar to the NoTg, whereas the sedentary group did not progress in a similar way.

In conclusion, physical exercise demonstrated certain neuroprotective effects in 3xTg-AD mice, confirming its value as preventive therapy against the aging-linked cognitive impairment.

Nuclear TAU in aging neurons of human PNS

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Human tau is a MAP encoded by a single-copy gene that produces three transcripts of 2, 6 and 9kb. While the 2kb transcript has been ubiquity identified in nucleus, the 6 and 9kb transcripts are expressed on CNS and PNS respectively, and also in non-neuronal tissues. The tau activity is regulated by phosphorylation. Hyperphosphorylated tau in determined epitopes is the main constituent of cytoplasm paired helical filaments characteristics of degenerative pathologies like Alzheimer´s disease (AD) and Inclusion-body myositis (IBM), both age-associated disorders.

We have study the interaction of some tau phospho-antibodies with nuclear DNA in neurons of peripheral nervous and muscle fibers in 5 biopsy samples of cancer and young and aging appendix. Only AT180 (p-T231/pS235) has nuclear landmark. In previous studies, Tau 1 has been located in nucleoli of neuronal cells and AT8 in the global chromatin of Alzheimer´s neurons, in the CNS. Besides, our initial results show that in neurons of peripheral nervous of all histological samples AT180 is localized in cytoplasm, like the others phospho-antibodies tested, but also is in nucleus interacting with the

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global-chromatin, however the degree and form of the staining is markedly different. A similar result has been obtained in muscle fibers.

We propose that hyperphosphorylated tau plays a role in global-chromatin dynamic of CNS and PNS aging neurons.

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Excitotoxicity in Alzheimer's disease is mediated by the Inactivation of APC/C-Cdh1 E3 Ubiquitin Ligase

Fuchsberger. T[1]**,** Lloret. A, Giraldo. E, Monllor. P, Vina. J

Excitotoxicity is a process that results in injuries to nerve cells by excessive stimulation of L-glutamate or other neurotransmitters. A growing body of evidence suggests that perturbations in systems using glutamate plays a major role in neurodegenerative diseases like Alzheimer's disease (AD), but the molecular mechanisms of this phenomenon are still not fully understood. Here we describe a new signaling pathway, how the AD-related peptide Aβ, causes enhanced generation of glutamate in neurons. This mechanism involves cdh1, the activator protein of the anaphase-promoting complex / cyclosome (APC/C), an E3 ubiquitin ligase, which plays an important role in post-mitotic neurons. Our results show that Aβ treatment decreases the protein level of cdh1 in primary neuron culture. This leads to a subsequent accumulation of glutaminase, a recently identified degradation target of APC/C-Cdh1, an enzyme that converts glutamine to glutamate. We

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showed that Aβ enhances glutamate generation and that this is mediated by deactivation of APC/C-Cdh1. The neurotransmitter increases in the extracellular culture medium and this causes an enhanced Ca^{2+} influx into neurons. The increase of glutamate caused by Aβ can be attenuated using a glutaminase inhibitor it is completely abolished when using medium that does not contain glutamine, the substrate of glutaminase. Analysis of transgenic APP/PS1 mice showed a decreased level of cdh1 compared to wildtype mice and glutaminase is increased in the transgenic animals. These results indicate that excitotoxicity mediated by the ubiquitin ligase APC/C-Cdh1, which is important to explain AD pathophysiology.

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Determination of biomarkers in plasma of patients suffering from mild cognitive impairment or Alzheimer's disease

Giraldo. E[1], Lloret. A, Badia. MC, Fuchsberger. T, Monllor. P, García de la Asunción. J, Alonso. D, Viña. J

Alzheimer's disease (AD) is the most common form of dementia. Finding new biomarkers of early AD has turned into a priority for the scientific community. The aim of this study is to identify bio-markers of mild cognitive impairment (MCI) and AD in blood samples and cerebrospinal fluid (CSF).

Determination of Aβ-42, total tau and p-tau in CSF have been included as criteria for diagnosis of AD. Samples of 12 healthy persons (controls), 12 patients with MCI and 12 AD patients, were analysed. CSF was extracted by lumbar puncture, and blood samples were taken to obtain plasma and serum. Levels of Aβ-42, total tau and p-tau, clusterin, regulator of calcineurin 1 (RCAN1), Protein kinase RNA-activated (PKR), calcineurin, receptor of advanced glycation end-products (RAGE), and ApoE were determined. Patients diagnosed for MCI or AD showed lower levels of Aβ-42 in the CSF and increased levels of tau total and p-tau. AD patients had lower protein levels of clusterin and increased levels of RAGE. In contrast, patients with MCI displayed increased levels of RCAN. Both, patients of AD and MCI showed decreased levels of PKR. Finding blood biomarkers will be promising for early diagnosis of AD and in the development of successful therapies. Determination of Aβ-42, total tau and p-tau correlates with the clinical diagnosis of patients. Clusterin and RAGE could be used as biomarkers for AD, while RCAN seems to be an early biomarker of AD. Both, clusterin and RAGE seems to be involved in Aβ clearance since they correlate with Aβ-42 levels.

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The role of the liver in Alzheimer's disease

Bonet-Costa. V[1], Mas-Bargues. C[1], Dromant. M[1], Inglés. M[2], Gimeno-Mallench. L[1], Gómez-Montaner. P[1], Gambini. J[1], Borrás. C[1], Viña. J[1]

Alzheimer's disease (AD) is a neurodegenerative disorder that causes progressive loss of cognitive functions, and is associated with aging and oxidative stress. The liver serves to detoxify pathological substances from bloodstream. One of them is amyloid-β peptide (Aβ). It can reach the liver because the action of lipoprotein receptor-associated protein 1 (LRP1), which mediates the transport from brain to blood, and from blood to liver. Receptor of advanced glycation products (RAGE), mediates Aβ transport from blood to brain. Therefore, hepatic care is fundamental for an integral AD treatment.

We used the double transgenic mice for AD APPswe/PS1dE9, which develops Aβ plaques with aging. We worked with both wild type and transgenic males of different ages, 3-5, 10-13 and more than 20 months (n=5), to follow age-associated changes. We analyzed oxidative stress parameters in liver, such as the rate of mitochondrial H2O2 production, protein oxidation (carbonylation) and lipid peroxidation (malondialdehy-

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de). We have also determined blood concentration of Aβ40, LRP1 in brain and liver, and RAGE in brain.

The results show how in transgenic mice, the different oxidative stress parameters decrease with aging, Aβ40 increases with aging, LRP1 decreases with aging in brain and liver, and RAGE in brain has no changes.

The decrease in LRP1 with age may explain the accumulation of Aβ40 in blood (it cannot get into the liver) and the lower oxidative stress in liver.

We can conclude that hepatic LRP1 is the main responsible for hepatic oxidative stress changes in AD.

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SESSION 6
Genetic and epigenetic regulation in aging

INVITED SPEAKERS

Analysis of gene expression in centenarians reveals specific regulation of apoptosis

Viña. J[1], Borras. C[1], Abdelaziz. KM[1], Serna. E[1], Gambinii. J[1], de la Fuente. M[2], García. AI[3], Sanchís. P[4], Belenguer. A[4], Avellana. JA[4], Rodríguez-Mañas. L[5], Matheu. A[3]

Centenarians not only reach exceptional longevity, they also evade age-related morbidities and exhibit "successful aging." In the present study using functional transcriptomic analysis (microarrays) we identified 1721 mRNAs expressed by centenarians but not by septuagenarians and young people. We next performed a sub-network analysis on these 1721 mRNAs and found six common "master regulator" genes: interferon (IFN)-γ (IFNG), T-cell receptor (TCR), tumor necrosis factor (TNF), SP1 transcription factor (SP1), transforming growth factor (TGF)-β1 (TGFb1) and IL-32.These six centenarian-specific genes were discovered related to four common genes such as Bcl-xL, Fas, FasL, and CCL5 (RANTES) whose functions converge. Intriguingly, Fas and FasL are involved in extrinsic, cellular surface receptor-mediated apoptosis whereas Bcl-xL inhibits intrinsic (mitochondrial) apoptosis. RT-PCR analysis of peripheral blood mononuclear cells revealed that centenarians indeed up-regulate Bcl-xL compared with septuagenarians and young people. Furthermore, caspase 8 activity was demonstrated higher in centenarians than septuagenarians and young people, suggesting that centenarians up- regulate the extrinsic pathway but down-regula-

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te intrinsic apoptosis. In vitroexperiments in mouse embryo fibroblasts (MEFs) demonstrated that cells overexpressing Bcl-xL display increased proliferation, decreased expression of p16Ink4a, p19Arf, and p21cipcell cycle regulators and diminished the activity of senescence markers β-galactosidase and Dcr2 compared with control MEFs. Moreover, expression of Bcl-xL significantly, bestowed cytoprotective effects against oxidative damage.

Hence Bcl-xLover-expresssion confers not only antiapoptotic but also anti-senescence effects. These results provide a glimpse as to how the very oldest persons in society achieve not only long lives but also healthy long lives.

Genomic predictors of mammalian longevity

Lehmann. G[1, 2], Muradian. KK[3], Fraifeld. VE[1]

Why do species differ in lifespan and what are the determinants of their longevity? Comparative studies on longevity in mammals revealed numerous variables that correlate with maximum lifespan (MLS). Yet, because of the intangibly intertwined biological relationships, only a limited number of the variables could be considered independent determinants of longevity. Using the methods of multivariate statistics, we found that four variables – mtDNA GC content, telomere length, body mass (BM) and body temperature (Tb) – have a significant and independent impact on MLS determination. Their combination exhibits extremely high fitting between predicted and observed MLS values and could explain almost 90% of the mammalian MLS variation. BM and mtDNA GC content display positive coefficients of regression while telomere length and Tb display negative coefficients, highlighting the role of potentially damaging and protective (stabilizing) factors in lifespan determination. The increase in BM and mtDNA GC content, reduction in telomere length and lower Tb

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could result in a higher genomic and metabolic stability, more efficient cellular homeostasis, and ultimately in increased longevity.

ORAL COMMUNICATIONS

Elucidating dual role of Wnt signalling pathway in age-regulation in Caernorabditis elegans

Lezzerini. M[1], **Budovskaya. Y**[1]

Although the role of Wnt signaling pathway during development is well studied, very little is known about the possible actions of Wnt signaling in natural aging. Our study focuses on Wnt signalling pathways and discusses how this genetic program orchestrates changes in the organism that could case aging. For the first time, we are using *Caenorhabditis elegans* as a model system to determine the role of Wnt ligands in aging. *C. elegans* has 5 Wnt proteins, *mom-2, egl-20, lin-44, cwn-1*, and *cwn-2*. We show that all five Wnt ligands are expressed and active past the development stages, and general Wnt activity increases, as worms grow old. The ligand *mom-2*/Wnt plays a major detrimental role in longevity, whereas the function of *lin-44*/Wnt is beneficial for long life. Interestingly, no evidence was found for Wnt signaling being involved in cellular or oxidative stress responses during aging, thereby suggesting that Wnt signaling regulates aging-intrinsic genetic pathways, opening a new research direction on the role of Wnt signaling in aging and age-related diseases.

We are using a system biology approach to reveal the molecular basis for the role of Wnt signaling pathway in aging in nematodes C. elegans by characterizing gene expression differences between young and old animals in variety Wnt signaling mutants, and then determining how these changes contribute to aging. This analysis revealed that Wnt signaling switches it function form regulating organismal growth and development, to mainly

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regulating cellular metabolism. In particular, blocking Wnt signaling alters the expression of fatty acid metabolism and oxidative phosphorylation pathways, the hallmarks of Warburg metabolism. We are validating these findings using untargeted metabolomics analysis of these mutants.

Methylation of human promoter ribosomal RNA genes is correlated with aging and aging phenotypes

Passarino. G[1], D'Aquila. P, Bellizzi. D, Montesanto. A

The transcription of the ribosomal RNA genes (rDNA) is a control point highly regulated in ribosome biogenesis [1]. This transcription is subject to epigenetic regulation in both physiological and pathological conditions, including cancer and Alzheimer disease [2]. In particular it is abrogated by the methylation of specific CpG dinucleotides located within the rDNA promoter [3]. It has emerged an age-related increase in rDNA methylation in several tissues of differently-aged mice. However, no data are yet available regarding a possible age related epigenetic regulation of human rRNA genes.

To fill this gap we investigated the age-related variability of methylation within the rDNA promoter region by Sequenom EpiTYPER assay in 667 samples collected from 22-105 years old human subjects. We found that methylation levels were higher in younger (<80 years) than in older (80+ years) subjects (0.319 vs 0.296, p=0.073).In the younger group we found that methylation levels significantly increased with age (p=0.0066), while no difference where detected for the older one. In addition, the variability in the methylation levels of the analyzed region also affected the functional decline of females individuals belonging to the younger groups. To our knowledge, this represents the first evidence about the correlation of the methylation of the rRNA promoter region with aging and functional decline that occurs during this complex process.

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Analysis of Age Effects on Gene Expression Using RNA-seq Data from Multiple Tissue

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Effects of aging on gene expression are diverse, manifesting on mean and variance, splicing, and environmental changes to genetic regulation (GxE). We analysed RNA-seq data from adipose, skin, whole blood, and lympho-blastoid cell lines (LCLs) from ~850 female adult twins from the TwinsUK cohort (39-85 years old) and found that 34% genes in all tested tissues changed in expression with age. Of those 5,224 genes affected by age, 8.3% were significant in two tissues with only 5 genes in common among the three primary tissues. Using fat methylome data available for 552 of the twins (Grundberg, 2013), we tested whether expression changes with age were also related with epigenetic changes and founded than 10.54% of the CpG markers tested were associated with age.

To further understand these changes in expression we investigated difference in expression within monozygous twin (MZ) pairs with age (discordance). We found 2 genes in adipose, 69 in skin, 1 in blood and 1 in

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LCLs where discordance was age-dependent. As MZ twins are genetically identical, the differences must be due to a changing environmental component. Decomposition of variance showed that age related genes had a larger genetic component, and that the sources of variation were highly tissue specific. To identify genetic sources of variation whose effects on gene expression changed with age we performed a genome-wide scan looking for age-genotype interactions (GxA). In summary, we produced a comprehensive description of how aging affects expression and its genetic control, observing that these effects are frequently tissue specific.

Genes and Life-style Factors in BELFAST Nonagenarians: Nature, Nurture and Narrative

Rea. M[1]

The number of the 'oldest old' is increasing. By 2050, 1 in 10 people in EU will be 80 years old and over. Increased '*lifespan*' needs to be accompanied by increased '*health span*'. Understanding how to 'Age Longer and Age Well' is a priority. In the **Belfast Elderly Longitudinal Free-Living Ageing STudy (BELFAST),** we assessed some genetic and life-style factors likely to contribute to 'successful ageing'.

Subjects over 80 years of age, who were apparently well, free-living in the community, mentally competent and living in the Greater Belfast area were invited and consented to be included in the BELFAST study for assessment of genetic, biochemical and nutritional markers together with a narrative life-style enquiry. Octo/nonagenarians in the upper tertile for weightt, Waist Hip Ratio and Body Mass Index were 40% more likely to have Blood Pressure in hypertensive range compared to those in lowest tertile. The ApoE4 gene associated with cholesterol carriage had a lower frequency 7.5% v 15.9% p=0.0008 in subjects <65 years of age while ApoE2 had significantly higher frequencies 12.2% v 8.3% P=0.047 in octo/nona-genarians compared to MONICA study subjects <65 years. There were

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age-related increases in pro-inflammatory cytokines including IL-6 with IL-6 gene GG polymorphism more frequent compared to CG phenotypes in a meta-analysis. The KIR haplotype B carriers associated with Natural Killer Cell function had higher pro-inflammatory profiles compared with A haplotype carriers. The mitochondrial J haplotype was more common in BELFAST nonagenarians compared to younger groups and was associated with lower blood pressure. Assessment of global methylation and P16 tumour suppressor methylation suggested that octo/nonagenarians had a more stable genomic pattern with some suggestion of association with folate and selenium status. In the associated narrative enquiry, 90 year olds identified nutrition, mental and physical activity and genetic factors as important in their good health and longevity.

The interaction between genes and lifestyle seems to be important in good quality ageing in BELFAST octo/nonagenarians. Both narrative enquiry and biological quantitative methodologies seem to suggest that avoiding cardiovascular disease, having a competent immunological system, a facilitatory genetic profile and good nutritional profile may be important in good quality ageing.

POSTERS

Analysis of single nucleotide polymorphisms in centenarians

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The study of human populations with extreme longevity (centenarians) is one of the major challenges facing scientists. Single nucleotide polymorphisms (SNPs), are nucleotide changes in the DNA sequence that may affect both the structure and the regulation of protein.

The aim of this study was the identification of single SNPs and SNPs grouped by signaling pathways as a possible cause of extreme longevity.

We recruited 28 centenarians and 60 young and elderly individuals from Hospital de la Ribera, Alzira, Valencia, collecting whole blood for DNA isolation. Sequencing was performed using the AxiomTM Genotyping of Affymetrix®, analyzing 295.988 SNPs. After applying a series of filters and genetic quality controls, only 37.564 SNPs remained, for which the association study was performed using logistic regression analysis. Moreover the SNPs genotyped, which belonged to genes located in 117 signaling pathways, selected from three databases, were grouped in the different pathways.

The results show a group of 108 SNPs with a p value less than 0.05, with twelve SNPs with a p value less than 0.001. Seventeen of the 108 SNPs, are located in fourteen different genes among ten signaling pathways.

The study of SNPs characteristic of centenarians could be a new tool to find genes and signaling pathways involved in healthy aging.

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The role of genes in development of ageing. Are genes masters or slaves?

Khalyavkin. A[1, 2]

One of the modern view postulates that senescence is a consequence of age-dependent changes in expression of set of genes, products of which are partially involved in a big and cross-talking net of specific signal transduction pathways controlling of ageing process. From this point genes are masters in development of ageing. But we know that activity of gene is dependent on the local regulation by microenvironment in which it is expressed. In turn, the concrete features of this microenvironment depend on changes in control systems due to signals from sensors accepting external influences of habitat. Potentially agelessness of the somatic stem cells and an evaluation of theoretically attainable minimal rate of ageing realizable in environmental conditions, which induce organisms to function optimally, as well as an analysis of the all findings taken as a whole, shows that they are compatible with the hypothesis that adequate living conditions are conducive to a significant deceleration of the human ageing process. Moreover the newest findings indicate that in adequate internal milieu even senescent mitochondria, stem cells, tissues etc. can restore lost status quo.

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From this point genes are slaves in development of ageing while external signals and cues from habitat are the masters.

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Survival transcriptome in the coenzyme Q deficiency syndrome is acquired by epigenetic modifications

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The coenzyme Q deficiency syndrome is a very rare condition that causes mitochondrial dysfunction and includes a variety of state clinical presentations as encephalomyopathy, ataxia and renal failure. We sought to set up what all have in common, and investigate why coenzyme Q supplementation reverses the bioenergetics alterations in cultured cells but not all the cellular phenotypes. To do that, (1) we analyze the common gene expression profile in primary cell cultures of dermal fibroblasts from patients suffering any of the clinical presentation, (2) we determine why coenzyme Q treatment restored respiration but not all the clinical phenotypes, and (3) we investigate the stable genetic cause responsible for the survival adaptation to mitochondrial dysfunction owing to coenzyme Q deficiency. Our results demonstrated that the survival transcriptome is acquired by epigenetic modifications of chromatin, including DNA-demethylation and histone modifications in specific residues. Also, these results approach to

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explain the incomplete recovery of clinical symptoms after coenzyme Q treatment and could establish the basis for healthy aging for these patients.

SESSION 7
Immunosenescence

INVITED SPEAKERS

Immunity and Ageing: Causes and consequences of immunosenescence in humans

Pawelec. G[1]

Infectious disease is both more frequent and more severe in the elderly, and the efficacy of prophylactic vaccination is commonly decreased. The elderly are particularly susceptible to newly-emerging or newly-encountered pathogens but persistent herpesviruses such as Varicella Zoster and Cytomegalovirus (CMV) also reactivate more frequently. These observations suggest some degree of immune compromise which may be reflected in the distribution of immune cells and antibodies in the peripheral blood. There is a great deal of heterogeneity in disease susceptibility and response to vaccination in humans such that establishing sets of immune biomarkers that reflect immune competence of the individual would be useful in this context to predict responsiveness to vaccination and susceptibility to infectious disease. This would allow better selection of prophylaxis and treatment options in the elderly, and facilitate understanding of the mechanistic basis of immunosenescence. Defining relevant age-associated alterations and identifying reliable biomarkers for monitoring clinically-relevant immune status in the elderly is therefore crucial. Ideally, this would mean performing longitudinal studies assessing innate and adaptive immunity, and correlating multiple factors with morbidity and mortality at follow-up. Here, I will discuss our data from the Swedish OCTO/NONA studies (which gave rise to our concept of the "Immune Risk Profile"), the Newcastle 85+, Leiden 85+ and Leiden Familial Longevity Studies, the US NHANES survey, the Belfrail Study and the new Berlin BASE II study. From these, we are beginning to assemble a more complete picture of clinically-relevant immune signatures which may inform interventions to prevent or reverse immunosenescence.

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NK cell subsets: Remodeling by aging and CMV

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NK cells are key players in the innate immune response against virus infection and tumors. Age-associated changes in NK cell phenotype have been previously reported that can be responsible of functional NK cell deficiency. Here we describe the current knowledge on age-associated changes in NK cells and the role of persistent CMV infection in configuring NK cell compartment in the elderly. NK cell frequency and NK cell subsets defined by the use of CD56 and CD16 markers and the expression of NK cell differentiation markers has been studied in CMV seropositive and seronegative young donors and on seropositive elderly donors. CMV seropositivity in young individuals does not significantly affect NK cell subsets. In contrast the percentage of NK cells is observed in elderly donors, all of them CMV seropositive, was significantly increased when compared with young CMV seropositive subjects. In addition a decrease in the percentage of CD56bright NK cells, either fully immature CD16 negative or CD16+ and an increase in the CD56−CD16+ subset were also found in the elderly. CMV seropositivity either in healthy young or elderly individuals is associated to the expression of CD94/NKG2C dimers and high expression of CD57on the CD56dimCD16+ NK cell subset. CD56−CD16+ NK cells, which are expanded in the elderly, show an increased expression of CD94/NKG2C and CD57 in CMV seropositive young donors when compared with CMV seronegative young individuals. In conclusion these results support that shaping of NK cell subsets in the elderly are related not only with age but also with exposure to pathogens, especially CMV.

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ORAL COMMUNICATIONS

Aging markers in the neuroimmune communication

Martínez de Toda. I[1], Vida. C, De la Fuente. M

Under stressful conditions, the expression of Hsp70 is induced, which acts as a cellular defence mechanism. An impairment in this induction has been related to aging whereas an increase has been related to longevity. Nevertheless, it is still not known if the basal levels of Hsp70 can be indicative of the aging rate of a tissue.

The aim of the study was to quantify the basal levels of Hsp70 in the immune system in naturally long-lived mice in relation to mice from other ages, and in a model of prematurely aging mice based on their spontaneous exploratory capacity in a T-maze. Adult animals, which took longer to explore the maze (prematurely aging mice: PAM) showed anxiety-like behavior, premature immunosenescence and a shorter life span than the nonprematurely aging mice (NPAM) of the same chronological age.

Adult, mature, old and long-lived (6, 12, 18 and 30 months old, respectively) ICR-CD1 female mice as well as PAM (6,12 and 18 months old) were used for this study and the Hsp70 basal levels were assessed in spleen and peritoneal leukocytes using an ELISA method.

The results showed that the Hsp70 levels in immune cells from old mice were lower than those in adults, whereas in long-lived mice were comparable to those in adults. In PAM these levels were similar to those in old mice. These results (Hsp70 levels preserved in long-lived animals and decreased in PAM) show the important role of Hsp70 in the rate of aging and for achieving a healthy longevity.

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The calpain-calpastatin system in human lymphocyte immunosenescence in view of the CALPACENT project

Mikosik. A[1], Jasiulewicz. A[1], Frąckowiak. J[1], Ruckemann-Dziurdzinska. K[2], Foerster. J[3], Schetz. D[4], Martorana. A, Buffa. S, Bulati. M, Collona-Ramana. G, Caruso. C, Witkowski. JM[5]

Centenarians form a selected group of long living humans; their longevity is conceivably associated with fitter immune systems. The calpain-calpastatin system (CCS) in the lymphocytes and many other cell types contains two neutral, cytoplasmic proteases (1- and 2-calpain) and their endogenous inhibitor – calpastatin. Their property of cleaving only relatively few specific sites in the proteins and peptides, as well as their list of substrates, makes them potentially important regulators of the immune response. Thus it is feasible that the system may take part in the process of immune system ageing; consequently, a hypothesis of different activity of the CCS in the immune cells of centenarians (as compared to younger healthy elderly) is explored in this project.

Thus, the detailed aim of the Polish-Italian CALPACENT project is to establish the role of CCS system function for the effective immune response and of its malfunction as a potential participant in the mechanisms of the immune cells' ageing.

We assess the numbers and activation of lymphocyte populations and the amount and the activities of CCS in the various subpopulations of peripheral blood cells from healthy Polish and Italian: centenarians, elderly and young people. We demonstrate the influence of calpain on the cell pro.

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liferation as well as the consequence of inhibition of calpains in the in vitro lymphocyte culture. Our results suggest the differences in the expression of CCS members between the different lymphocyte populations (including the CD4+, CD8+, CD19+) and in their activity, bearing on the cells' functions.

HSP70 Basal levels in immune system are preserved in long-lived mice and decreased in prematurely aging mice

Martínez de Toda. I, Vida. C, De la Fuente. M

Under stressful conditions, the expression of Hsp70 is induced, which acts as a cellular defence mechanism. An impairment in this induction has been related to aging whereas an increase has been related to longevity. Nevertheless, it is still not known if the basal levels of Hsp70 can be indicative of the aging rate of a tissue.

The aim of the study was to quantify the basal levels of Hsp70 in the immune system in naturally long-lived mice in relation to mice from other ages, and in a model of prematurely aging mice based on their spontaneous exploratory capacity in a T-maze. Adult animals, which took longer to explore the maze (prematurely aging mice: PAM) showed anxiety-like behavior, premature immunosenescence and a shorter life span than the nonprematurely aging mice (NPAM) of the same chronological age.

Adult, mature, old and long-lived (6, 12, 18 and 30 months old, respectively) ICR-CD1 female mice as well as PAM (6,12 and 18 months old) were used for this study and the Hsp70 basal levels were assessed in spleen and peritoneal leukocytes using an ELISA method.

The results showed that the Hsp70 levels in immune cells from old mice were lower than those in adults, whereas in long-lived mice were comparable to those in adults. In PAM these levels were similar to those in old mice. These results (Hsp70 levels preserved in long-lived animals and

decreased in PAM) show the important role of Hsp70 in the rate of aging and for achieving a healthy longevity.

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Peptide KEDW regulates B-cell differentiation during ageing of pancreatic cells

Khavinson. VKh[1, 2, 3], Tarnovskaya. SI[2], Linkova. NS[1, 4]

The addition of H-Lys-Glu-Asp-Trp-NH2 (KEDW) peptide to the «old» pancreatic cell culture leads to an increase in expression of the following differentiation factors genes: PDX1, NGN3, PAX6, FOXA2, NKX2-2, NKX6.1, PAX4, which participate in maturing of insulin-producing beta cells. According to results obtained with physical methods (UV–visible absorption, circular dichroism, viscosimetry) and molecular modeling the peptide binds to DNA. This process takes several hours and involves practically no electrostatic forces. As a result a complex formation is developed, which takes place in the major DNA groove with participation of a nitrogenous base and the peptide. The experimental and theoretical data obtained serve to provide a 3D model interaction of the peptide with respective DNA site and are indicative conformation of stable DNA-peptide complex. We suggest that the KEDW peptide activates expression of differentiation factors in senescent pancreatic (endocrine) cells due to specific binding with the regulatory elements of corresponding genes.

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Key words: KEDW peptide, DNA-peptide complex, diabetes mellitus, pancreas, gene expression, molecular modeling, regulation of transcription, cell senescence.

Senescence and growth-regulatory function of immune system

Khalyavkin. A[1, 2]

Many gerontologists believe that early involution of the thymus points to ageing program. Immunological theory of vertebrates' ageing suggests that the age-related reduction of organism's resistibility occurs due to early and genetically programed involution of immune system. Comparison of age-related change in force of mortality with age-related change of immune system activity showed a negative correlation between these dependencies, which indirectly justify this approach. However the reason of early reduction of immune activity in ontogenesis is not clear outside the ideas and concepts of noninfectious immunology. According to these concepts the vertebrate's immune system is primarily designed for immune surveillance over the initial tumor emergence and for tissue-specific regulation of cell proliferation in ontogenesis and during physiological and reparative regeneration of organs and tissues. Natural anti-tissue auto-antibodies are main effectors of such regulation. For this reason the number of histocompatibility genes (MHC-genes) equals the number of all cell types (~220) while the number of genes for variable part of antibodies (V-genes) equals the number of all proliferative-competent cell types (~100). For the same reason maximal rate of growth which is usually observed at prepubertal period coincides with maximal thymus index and with maximal number of immunoglobulin-secreting cells as well as with minimal force of mortality during ontogenesis. We proposed a quantitative model which united immunological, cancer and growth-regulation characteristics of individual.

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Indeed the dependencies of oncological status and finite weight of mice of various lines from their immunological parameter (thymus index) received in the model are compatible with experimental data.

Resveratrol reduces inflammatory markers in old mice liver

Tung. BT[1, 2,3], Rodríguez-Bies. E[1], Talero. E[2], Motilva. V[2], Navas. P[1], López-Lluch. G[1]

Inflammation is a hallmark of aging. Among the interventions delaying aging, caloric restriction, resveratrol (RSV) and exercise have shown the highest success. We investigate here the effect of RSV on inflammation markers in liver of old male C57BL/6J mice. Levels of IL-1β, IL-6, IL-10, IL-17 and TNF-α were evaluated by ELISA in young (8 months), mature (18 months) and old (24 months) liver mice. Levels of pro-inflammatory cytokines IL-1β, IL-6, IL-17 and TNF-α and also their respective mRNA increased in old mice liver. This increase was reversed by RSV in the case of IL-1β and TNF-α only in old mice liver but not in young neither in mature mice. This reduction was also found at the mRNA level. Levels of mRNA of the components of NALP-3 inflammasome, ASC, CASP-1, NALP-1 and NALP-3, also showed an age-dependent increase that were reversed by RSV. Protein levels of the active form of CASP-1 were also higher but also were reversed by RSV. Our study confirms that aging is accompanied by an increase in the proinflammatory pattern in liver and that RSV reduces this pattern in old mice liver.

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SESSION 8
Coenzyme Q in aging

INVITED SPEAKERS

Reduced coenzyme Q10 (ubiquinol-10) activates mitochondrial functions and decelerates senescence in senescence-accelerated SAMP1 mice and HepG2 cells

Higuchi. K[1], Tina. G[1], Xu. Z[1], Kubo. H[2], Hosoe. K[3] and Sawashita. J[1, 4]

Our studies have revealed significantly delayed senescence in Senescence-accelerated mouse prone-1 (SAMP1) mice given supplementation with reduced form of coenzyme Q10 (ubiquinol-10), but the mechanism of action of this effect has remained unclear1, 2). Here, we report that dietary ubiquinol-10 supplementation prevents age-related decreases in the expression of NAD+-dependent protein deacetylases (SIRT1 and SIRT3), which results in the activation of peroxisome proliferator-activated receptor coactivator 1α (PGC-1α), a major factor that controls mitochondrial biogenesis and respiration, as well as superoxide dismutase 2 (SOD2) and isocitrate dehydrogenase 2 (IDH2), which are mitochondrial antioxidant enzymes. Ubiquinol-10 supplementation prevented age-related decrease in mitochondria complex I activity and numbers of mitochondria and age-related increase in oxidative markers.

Furthermore, ubiquinol-10 may increase levels of cAMP by activating adenylate cyclase (AC) and repressing phosphodiesterase (PDE) that, in

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turn, activate cAMP response element-binding protein (CREB) and AMP-activated protein kinase (AMPK) in HepG2 cells3). Ubiquinol-10 suppressed the staining of senescence-associated β-galactosidase (SA β-gal) in SAMP1 mouse embryonic fibroblast (MEF) cells.

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Q prevention of accelerated cardiac aging

Hargreaves. IP[1], Tarry-Adkins. JL[2], Fernandez-Twinn. DS[2], Chen. JH[2], Martin-Gronert. MS[2], McConnell. JM[2], Ozanne. SE[2]

Nutritionally induced Low birth weight followed by accelerated postnatal growth has been reported to increase the risk of cardiovascular disease. Although the mechanisms underlying such nutritional programming are yet to be elucidated, increased oxidative and nitrosative stress leading to accelerated cellular aging have been proposed to play an important role. We have previously reported that animals that are born small as the result of maternal protein restriction, but grow up rapidly during lactation demonstrate increased oxidative and nitrosative stress, up-regulation of

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base excision repair machinery and increased DNA damage in heart tissue by weaning. These results indicate that increased oxidative stress leading to DNA damage is a very real consequence of suboptimal nutrition in utero and accelerated postnatal growth and therefore may be an important therapeutic target. Using an established rodent model of low birth weight and catch up growth we assessed whether post weaning dietary supplementation with coenzyme Q_{10} (CoQ_{10}), an electron carrier in the mitochondrial respiratory chain as well as a potent antioxidant was able to rescue the detrimental effects of nutritional programming on cardiac aging. Post CoQ_{10} supplementation of the offspring postnatal diet was found to correct the cardiac cellular stress, telomere shortening, antioxidant defence alterations and cellular senescence and apoptosis, thereby protecting against premature cardiovascular aging. These findings suggest a potential for postnatal CoQ_{10} supplementation to reverse the deleterious phenotypes of developmental programming and thereby provide an insight into a potential translatable therapy to prevent cardiovascular disease in at risk humans.

Ptc7p modulates yeast bioenergetics and lifespan through the regulation of both coenzyme Q biosynthesis and mitophagy

González-Mariscal. I, Martín-Montalvo. A, Navas. P, **Santos-Ocaña. C**[1]

Coenzyme Q (CoQ) in yeast depends on a multiprotein complex in which Coq7 protein has both catalytic and regulatory functions. Coq7p modulates the levels of CoQ through a phosphorylation cycle where dephosphorylation of three amino acids by the mitochondrial phosphatase Ptc7p increases the levels of CoQ. The conversion of these three amino acids (Ser/Thr) to alanine leaded to a permanently active form of Coq7p that significant-

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ly increased the levels of CoQ up to 250%. However, this modification induced a decrease in the mitochondrial respiratory chain activity, a lower resistance to oxidative stress, an increase of the endogenous production of ROS, an accumulation of carbonylated proteins, and a significant shortening of chronological life span (CLS) compared to the wild strain. A null mutant strain of the Ptc7p phosphatase showed a decrease in the synthesis of CoQ associated to a significant shortening of the CLS that was not reversed after adding exogenous CoQ. Overexpression of Ptc7p not only restored normal levels of CLS but also increased mitophagy in a wild type strain. This finding suggests an additional Ptc7p function besides its regulation of CoQ biosynthesis. An effect on autophagy was discarded but the absence of Ptc7p prevents mitophagy activation in conditions of nitrogen deprivation. We show here that Ptc7p modulates the adaptation of yeast to a respiratory metabolism by dephosphorylating Coq7p to supply newly synthesized CoQ but also activating mitophagy to remove defective mitochondria at stationary phase to guarantee proper CLS in yeast.

Coenzyme Q adaptations to aging, calorie intake level and dietary fat source

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Calorie restriction (CR) improves health-span and extends lifespan in many animal models partially by optimizing mitochondrial functions. A decrease in mitochondrial long-chain PUFAs may contribute to the anti-aging effects of CR. Thus, dietary fatty acids could influence life span in CR animals. Coenzyme Q (Q) is a lipid-soluble electron carrier and antioxidant. Very little is known about the effects of aging, CR and dietary fat on Q. We established two groups of mice fed lifelong diets containing soybean oil, either *ad libitum* or under CR, and two additional CR groups fed diets containing lard or fish oil. Q levels and Q biosynthesis transcripts (*COQ* mRNAs) were measured in liver, hindlimb skeletal muscle and kidney. Mitochondria ultrastructure and dynamics markers were studied in liver. CR abolished age-induced changes of Q levels in liver and skeletal muscle from mice fed PUFA-enriched diets. These tissues adapted to a PUFA-enriched CR diet by increasing Q levels and decreasing Q_9/Q_{10} ratio. The effect of CR on fission proteins in liver was exacerbated by lard and diminished by fish oil, even while liver mitochondria were largest in CR-lard, but differences were attenuated by aging. Q levels in kidney were dramatically decreased in young CR animals fed a fish oil-enriched diet but differences were also attenuated by aging. Fish oil-enriched diet enhanced overall abundance of *COQ* mRNAs. We detected a substantial increase of *COQ8* and a decrease of *COQ2* in kidney from old animals. Aging and dietary factors as CR and fat source modulate the Q system in mouse tissues.

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POSTERS

Age-related changes in genes expression of mitochondrial biogenesis and inflammasome in Fibromyalgia are restored by CoQ_{10}

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Objective: During aging several pathological processes lead to the deterioration of numerous physiological functions which will influence age-related pathologies. Inflammation and impaired mitochondrial biogenesis-related process have been widely described in aging. Recently, we have demonstrated that age-related changes in gene expression are manifested earlier in several Fibromyalgia (FM) patients (1). In this study, we hypothesized that some age-related changes in gene expression may be restored by CoQ_{10} treatment.

Material and Methods: We carried out a randomized, double-blind, placebo-controlled trial to evaluate age-related changes in gene expression after two month of CoQ_{10} supplementation (300 mg/day) or placebo on 20 FM patients. PGC-1α, Tfam, Nrf1, SOD2, and NLRP3 transcript levels were analyzed using real-time PCR analysis.

Results: Data analysis revealed significant transcriptome changes in all genes after CoQ10 treatment which was not observed in placebo group. CoQ10 induced an interesting reduction in the age related descent of mitochondrial biogenesis genes and a reduction in the activation of inflammasome gene NLRP3.

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Conclusions: Our results suggest that CoQ_{10} could have a potential role in the modulation of transcriptome changes associated with accelerated aging. Given the complexity of FM, more studies will be necessary to confirm this hypothesis. However, we think these studies could be extrapolated to other aging conditions in order to plan new interesting projects.

1. Alcocer-Gómez E, et al. Aging-related changes in inflammatory and LKB1/AMPK gene expression in fibromyalgia patients. CNS Neurosci Ther. 2014; 20:476-8.

Effect of kaempferol, a positive regulator of SIRT3, on coenzyme Q system

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Aging is characterized by a progressive loss of physiological integrity (Cell, 2013. 153:1194). The best characterized non-genetic intervention to significantly slow aging is calorie restriction (CR), which also delays the onset of numerous aging-associated diseases (Mol Aspects Med, 2011. 32:159). This ability of CR to retard aging is dependent, at least in part, upon activation of the sirtuins, a family of NAD+-dependent histone/protein deacetylases (J Clin Invest, 2013. 123:973). Mammals have seven sirtuins which are found in nucleus (SIRT1, SIRT6 and SIRT7), cytosol (SIRT2) and mitochondria (SIRT3, SIRT4 and SIRT5). SIRT3 is the major mitochondrial deacetylase, which regulates mitochondrial electron transport chain activity activating complex I, II and III (Trends Biochem Sci, 2010. 35:669). Moreover, some studies support that SIRT3 knockout mice are more sensitive to oxidative stress (Mol Cell, 2011. 42:561). We have hypothesized that coenzyme Q (Q) function, due to its essential role as electron carrier and as antioxidant (Mitochondrion, 2007. 7 Suppl:S41), could be regulated by sirtuins. Our study is focused on the development of an in vitro model to elucidate whether

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SIRT3 could modulate the Q system and its biosynthesis. Hepa 1.6 cells were cultured in the absence or in the presence of kaempferol, a flavonol that upregulates SIRT3 (Biochemistry, 2010. 49:304). Our results show that kaempferol, possibly by regulating SIRT3, could influence the Q system, which can give novel insights to further understand how sirtuins play such an important role in the regulation of mitochondrial metabolism.

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Comparing coenzyme Q_{10} levels in plasma and peripheral blood mononuclear cells in community-dwelling elderly-people: Relationship between functional capacity, body mass index and implications for welfare.

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Besides its prominent role in cell bioenergetics, Coenzyme Q has also been showed in recent studies having important antioxidant properties both in cell membranes and plasma, and also having a prominent part as a preventive agent for several aging-related diseases. However, to date no clear data on the levels of plasma Q during aging are available. A previous study was performed in our laboratory with a volunteer group of healthy community-dwelling people, which were subjected to different tests of the Functional Fitness. We measured anthropometric characteristics, plasma Q_{10} and oxidative stress markers in order to establish a relationship between functional capacity and body mass index with plasma coenzyme Q_{10}. We have lately extended this study measuring these relationships with

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Q_{10} levels in cryopreserved Peripheral blood mononuclear cells (PBMC), and we are showing a combined and integrated view of these studies in our poster. Our results suggest that while plasma Q_{10} levels seem to be related to cardiovascular capacity, PBMC Q_{10} levels can be more precisely correlated to muscle strength. Our last Q_{10} measurements in monocytes reveal a streaking similarity with similar studies correlating muscle Q10 levels and functional capacity, which ultimately agrees with the notion that muscle strength and aerobic capacity are opposing from the point of view of sports performance. Taken together, these results highlight the usefulness of the differential measurement of coenzyme Q in blood samples, separating plasma and monocyte fractions, in order to get a combined picture of how the degree of physical activity is affecting health and welfare.

Caloric restriction and aerobic exercise produce age-dependent effects on ubiquinone-dependent activities in mice muscle

Rodríguez-Bies. E[1], Rodríguez-Hernández. MA, Navas. P, López-Lluch. G

Aging affects many biochemical, cellular and physiological processes in the organisms. Oxidized macromolecules accumulate in many age-associated diseases. Coenzyme Q (Q) is one of the main molecules involved in metabolic and antioxidant activities in cells. Q-dependent antioxidant activities are importantly involved on the protection of cell membranes against oxidation. Many studies have shown that Q levels decay in most of the organs during aging. However, in our study no changes in Q levels were found in old animals in comparison with young animals. On the other hand, caloric restriction based on every-other-day feeding procedure and physical exercise were able to increase Q levels in muscle but only in old and not in young animals. Probably, this effect prevented the increase in

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lipid peroxidation found in aged animals and also protein carbonylation. Further, Q-dependent antioxidant activities such as NADH-Cytochrome b_5 reductase and NQO1 were also modulated by both, exercise and EOD. Taken together, we demonstrate that exercise and dietary restriction as EOD procedure can regulate endogenous synthesized Q levels and Q-dependent antioxidant activities in muscle preventing oxidative damage in aged muscle.

1. E. Rodríguez-Bies, P. Navas, G. López-Lluch. Age-dependent effect of every other day feeding and aerobic exercise in ubiquinone levels and related antioxidant activities in mice muscle. J. Gerontology A. In press (2014)

Coenzyme Q modulates longevity and the oxidative stress that is ongoing with aging in *Drosophila melanogaster*

Fernández-Ayala. DJM[1, 2], Sanz. A[3, 4]; Stefanatos. RKA[4]; Jacobs. HT[4]; Navas. P[1, 2]

The Mitochondrial Free Radical Theory of Aging described that longlived individuals or species should produce fewer mitochondrial Reactive Oxygen Species (mtROS) than shortlived individuals or species. Because coenzyme Q (CoQ) is directly involved in the electron transference for both ATP production inside mitochondria and free radicals removal in all the cell membranes and lipoproteins (1), we analyzed different strains of Drosophila melanogaster which showed a wide-range in longevity (2), to set up if CoQ could modulate the longevity and the oxidative stress that is

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ongoing with aging. As expected, the long-lived strain showed less mtROS production whereas the short-lived ones produced higher levels of mtROS. We found 3 different isoform of CoQ, named CoQ8, CoQ9 and CoQ10, in Drosophila, which differ in the length of its isopronoid tail. CoQ8 isoform was present in all the strains at a low level (around 5% of the total amount of CoQ). However, we found differences in the content of CoQ9 and CoQ10, which altered the proportion of these CoQ isoforms. The percentage of CoQ9 was higher in the long-lived strain, whereas CoQ10 percentage was increased in strains with higher mtROS production.

These results suggest that CoQ9 and CoQ10 are the responsible of the dual function of CoQ, being CoQ10 isoform the predominating one in an environment with higher antioxidant needs. Also, a possible modulation of CoQ10 level to alter the ratio of CoQ9 and CoQ10 could modulate longevity and the oxidative stress that is ongoing with aging, at least in Drosophila melanogaster.

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2. Sanz A, Fernandez-Ayala DJ, Stefanatos RK, Jacobs HT. Mitochondrial ROS production correlates with, but does not directly regulate lifespan in Drosophila. Aging (Albany NY). 2010; 2(4):220-3.

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