

Experts' opinion on current
approaches in anti-ageing
medicine and gerontology

BOOK OF ABSTRACTS

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developed. In the coming years, the perspective direction could be the development of new biomarkers, based mostly on biochemical and genetic methods, for short-term screening of such drugs. At present, cooperative studies on anti-ageing drugs and geroprotectors conducted in various laboratories could be promising.

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MEDITERRANEAN DIET AND LIFESTYLE: A PROVED APPROACH TO HEALTHY LONGEVITY

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Relevance. Aging is a multifactorial and progressive process, universal and irreversible, that takes place at different levels, affecting practically all living organisms. Human life expectancy increased extraordinarily during the last century worldwide. Currently, there is growing evidence that there are modifiable factors that contribute to ageing *per se*, and particularly to longevity (i.e. diet, physical and mental activity). These factors may interact with the ageing process and may alter the susceptibility of an individual to develop age-associated diseases. There is currently much promise in research that provides information about the underlying biology of ageing and longevity, which has unveiled possible interventions to slow the ageing process, including a healthy lifestyle in terms of nutrition, exercise, and smoking cessation, as well as new

discoveries that result from basic research. Diet is a major determinant of the ageing process itself and of the development of age-associated diseases. There is strong evidence of the influence of diet on the health status. Epidemiological and experimental evidence have emphasized the traditional diet from the Mediterranean basin as a possible contributor to longevity and good quality of life. After the first positive data from the Seven Countries Study, numerous investigations in different populations have confirmed a beneficial role for the Mediterranean dietary pattern on the reduction of overall mortality and mortality from cardiovascular diseases and cancer, hence, increasing longevity (1, 2). A meta-analysis including a total of over 1.5 million subjects confirmed a significant reduction in all-cause mortality associated to increased adherence to the Mediterranean diet (2). These investigations have over and over again confirmed that the Mediterranean diet is a model of healthy eating which contributes to a favourable health status and a good quality of life (3). The PREDIMED (Prevención con Dieta Mediterránea) study, a prospective randomized controlled trial, which included 7,447 volunteers aged 55 to 80 years at high cardiovascular risk, demonstrated that a Mediterranean diet supplemented with extra virgin olive oil or nuts significantly reduced major cardiovascular events after a median follow-up of 4.8 years (4). Using data from the SUN (Seguimiento Universidad de Navarra) project, a large, prospective cohort, we found that the risk of the combined outcome of incident cardiovascular events, incident diabetes incidence or all-cause mortality, was significantly lower with a higher adherence to Mediterranean diet, even after adjustments for total energy or weighting each item's evidence-based contribution to coronary heart disease protection (5).

What is Mediterranean diet? The Mediterranean diet is a balanced dietary pattern, high in foods of plant origin, which are rich in antioxidant compounds, vitamins and fiber, similar to that followed by traditional populations living in the Mediterranean basin during the post-World War II period. It is mostly based on whole bread and grains, extra virgin olive oil, legumes, fresh vegetables and fruits, nuts, fish, herbs and spices, and wine in moderate amounts. There is mounting evidence that this dietary pattern have remarkable effects reducing total mortality, cardiovascular mortality, and cancer-related mortality. The name "Mediterranean diet" was coined in the 60's by Ancel Keys, a researcher of

the University of Minnesota, who arrived in Italy after the World War II. He carried out a 20-year investigation exploring the effects of diet on the incidence of various diseases in some Southern Italian regions and Greece compared to the incidence of those diseases in the USA, Finland, Japan, the Netherlands, and former Yugoslavia. Less similar the dietary pattern used compared to the Mediterranean diet, the higher was the incidence of the diseases of "abundance", i.e., cardiovascular disease and some types of cancer (1). The definition of a single Mediterranean diet as a unique entity is not simple because there are countries with cultural, ethnical, religious and economic diversity in the region. However, the general pattern is similar and the use of extra virgin olive oil as the main source of dietary fat is universal in the basin, although it varies according to socioeconomic and demographic characteristics in some countries. The use of whole complex carbohydrates derived from wheat (bread, pasta, couscous) along with a minimal percentage of simple sugars, constitute 50-60 percent of the total caloric intake in the Mediterranean diet. The fat consumption (based almost exclusively on extra virgin olive oil) represents 25-30 percent, and protein intake (fish, legumes and meat) the remaining 10 percent of the total caloric intake.

Mechanisms of the Beneficial Effect of the Mediterranean Diet. The antioxidant and anti-inflammatory action of several components of this dietary model have been linked to its favourable effects regarding the prevention of degenerative diseases in old age. One of the most accepted mechanism to help explain the ageing process is the excessive mitochondrial production of free oxygen radicals (ROS: reactive oxygen species) with damage to cellular structure and subsequent chronic inflammation. Oxidative stress accumulates when prooxidants overwhelm the antioxidant defence mechanisms. Oxidative stress and chronic low-grade inflammation are involved in several age-associated conditions including hypertension/cardiovascular disease, type 2 diabetes mellitus, neurodegenerative diseases, including Alzheimer's and Parkinson's disease, dyslipidaemia, and cancer. ROS serve as precursors to the formation of oxidized LDL, essential for the formation of atherosclerotic plaques. Elevated ROS have been also associated with an increased expression of pro-inflammatory cytokines such as TNF-alpha, plasma activator inhibitor (PAI)-1 and interleukin-6.

These concepts have lead to seek for factors that may reduce ROS or contrast the cellular damage or enhance the repair mechanisms. Mediterranean dietary pattern includes a significantly large amount of plant foods rich in antioxidant compounds, which may help to explain its multiple benefits. Many of the components of the Mediterranean diet, including extra virgin olive oil, fresh vegetables and fruits, nuts, wine, and fish, contain molecules with anti-oxidant and anti-inflammatory properties such as monounsaturated fatty acids, omega 3 fatty acids, polyphenols, flavonoids, phyosterols, antioxidant vitamins, and antioxidant minerals and micronutrients. Among the components of this dietary model, the use of extra virgin olive oil as the main source of lipids has been linked to diverse favourable effects regarding the prevention of degenerative diseases in old age because of its antioxidants and antiinflammatory properties. Resveratrol, a potent free radical scavenger, contained in particular (but not exclusively) in red wine has been shown to modulate insulin secretion and action, and that it may extend lifespan in animal models. However, all the studies on the Mediterranean diet emphasize the effects of the whole dietary pattern with the combination of different nutrient-rich and antioxidant food rather than individual elements. In fact, the effects of the combination of different components of a balanced diet may potentiate the effect of single elements and may better help to explain the unquestionable evidence of the multiple benefits of this dietary pattern on longevity and against the development of age-associated diseases.

Conclusion. The Mediterranean diet is now considered worldwide as an ideal nutritional model, based on numerous scientific studies showing that people who follow this traditional lifestyle live longer and are healthier, with lower incidence of cardiovascular disease, obesity, atherosclerosis, diabetes, hypertension and cancer, compared to those adherents to a "western" type diet. The Mediterranean diet is balanced, high in foods of plant origin, which are rich in antioxidants, vitamins and fibers, and so rich in fresh fruits and vegetables, nuts, olive oil, fish, herbs and spices, and wine in moderate amounts. Adherence to a Mediterranean dietary pattern represents a promising option for the prevention of diverse diseases associated to aging contributing to maintain a favourable health status and a good quality of life.

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METABOLISM AMELIORATING REMODELING INDUCED BY ATMOSPHERE (MARIA)

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Relevance. Lower metabolic expenditure is more than often associated with declined rate of damage and longer lifespan. Such inverse relationship between metabolic and aging rates is well documented on numerous phylogenetic and ontogenetic models and could be a basic concept for many hypotheses of aging (wear and tear, waste accumulation, free radicals etc). Unfortunately, long-term decrease in metabolism in warm-blooded species remains an unresolved problem. At least all our efforts to decrease the metabolic rate and extend life span of laboratory animals by application of the known inhibitors of mitochondrial or nuclear replication, transcription, translation and uncoupling resulted in only marginal effects, presumably because of the adverse side-effects of the chronic usage of antibiotics/xenobiotics [Frolkis, Muradian, 1991]. Our recent findings, however, show that metabolic expenditures could be decreased/optimized by maintaining animals in hypercapnic/hypoxic atmospheres (HHA) [Timchenko et al., 2008; Muradian, 2013; 2015]. There is another important issue: basic life-supporting systems evolved and most part of their

evolution occurred in severe hypercapnia and hypoxia. Therefore, maintenance of modern animals in environments resembling primordial earth hypercapnic/hypoxic atmospheres could result in optimization of metabolic expenditures.

Methods. Experiments were performed on young (3-5 months) and old (22-25 months) male C57Bl/6 or female CBA mice. Effects of artificial atmospheres on viability in stress conditions, survival dynamics and lifespan were studied on drosophila (Oregon). Acute (3-4 hours) and chronic (several weeks) exposures of mice to HHA were studied. Acute HHA were modeled by keeping individual mice in hermetically closed glass jars. Chronic exposures were modeled by maintaining mice groups in boxes with regulated ventilation, so that animals themselves created necessary atmosphere (auto HHA). Specifically, mice were kept in auto HHA typical for the naked-mole rat (around 10% CO₂ and 10% O₂). In experiments with drosophila, flies were kept lifetime in hermetically closed 100 ml syringes with addition of 5-15% CO₂ to the air. Feeding medium and atmosphere were refreshed on every other day basis. In experiments with mice, food and water consumption, motor activity, real time PCR of hypoxia inducible factor (Hif1) and two hypothalamic genes associated with appetite stimulation (neuropeptide Y and agouti like protein), as well as oxygen consumption and CO₂ production rates (Vo₂ and Vco₂), body temperature, concentrations of the blood T3 and T4, glucose, total cholesterol, fatty acids, lactate and pyruvate were determined. Type 1 diabetes was modeled by injection of streptozotocin (40 mg/kg) during 5 days.

Results. It was found that dependence of Vo₂ and Vco₂ on partial pressure of CO₂ or O₂ (Pco₂ and Po₂) is characterized by three phases. At lower atmospheric concentrations (<2%), CO₂ induces paradoxical stimulation of Vo₂ and Vco₂. Middle range concentrations of CO₂ in the air (>2% and <5%) did not significantly change metabolic rate whereas Pco₂ higher than in the blood of mice (CO₂ > 5%) resulted in significant decrease of Vo₂ and Vco₂. Surprisingly, exposure to HHA typical for naked-mole rats was sufficient to induce analogous metabolic modifications in mice, i.e., decreased Vo₂ and Vco₂, food and water consumption without significant changes of the motor activity. Body surface temperature decreased by 2-4 °C, as it is typical for the naked-mole rats. To our best knowledge, MARIA is the only reliable mammalian model of chronic body temperature decrease. It is remarkable, that highly significant positive correlations were found