Olive oil, dietary fat and ageing, a mitochondrial approach

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SUMMARY

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Ageing represents a great concern in developed countries because the high number of people included in this group (indeed, a further increase in the rate of old people it is expected in the near future). Another important aspect concerning ageing is the number of pathologies related with this phenomenon like Alzheimer, Parkinson, diabetes, cardiovascular disease and cancer. According to the free radical theory of ageing and its further mitochondrial extension, ageing is the result of the oxidative insult to the organism throughout the life. Some of the damages are not entirely repaired and are accumulated, leading to organism malfunction. Such oxidative-stress related events are particularly important in mitochondria and specially at the mitochondrial DNA level (less protected and more prone to oxidation than nuclear DNA and with a not well established repairing system). Such mitochondrial damage directly affects to the cell energy delivery system, being that, at least in part, the explanation for the structural and functional impairments related to age. Oxidative stress is related with the fatty acid composition of membranes. The intake of a type of fat affects in a direct way the fatty acids and antioxidants composition of subcellular membranes (including mitochondrial membranes) and in an indirect way the susceptibility of the membrane to oxidation. Thus, if we build specific biological membranes according to particular types of fats, we would be able to positively affect the way and intensity in which different organs would age. This work hypothesis represents a new point of view in the investigation of ageing and might have important consequences. According to the above-mentioned premises, this work reviews the convenience to use virgin olive oil as dietary fat from the point of view of mitochondrial ageing.

KEY-WORDS: Olive oil; Sunflower oil; Mitochondrial electron transport chain; Aging; Mediterranean diet.

1. INTRODUCTION

Ageing is a phenomenon common to all multicellular organisms that has been described as an endogenous and progressive decay in the efficacy of the physiology of the organism after the reproductive phase (Halliwell and Gutteridge, 1999; Camougrand and Rigoulet, 2001; Barja, 2002). The importance of ageing resides in the high percentage of people over 65 five years (circa 20%), with a rise in the number of individual with more than 80 years.
Moreover, there is an increasing incidence of ageing-related pathologies like Alzheimer, Parkinson, diabetes, certain types of cardiovascular disease, cancer and the ageing-related degenerative-macular (the main cause of blindness in developed countries). The above-mentioned decay has been attributed to a genetic program present in all individual of the specie or to the stochastic accumulation of errors at the somatic cells that could lead to a progressive loss of the cell function (Camougrand and Rigoulet, 2001). Since 1840, life expectancy has increased in developed countries at a ratio of three months per year. A good new is that health has improved, but the health-related cost has been also increased, with a number or new diseases that almost did not exist a century ago (Halliwell and Gutteridge, 1999; Partridge and Gems, 2002). However, if ageing is deleterious for individuals and it exists all over the world, why does it happen?. The answer to that question might be that ageing is the side effect of something else. Thus, genes that delay ageing could do that by repression of the cause that generates the damage associated to ageing. A source of such type of damage is reproduction. Fertility is frequently reduced both evolutionary when ageing decreases as well as by the presence of punctual mutations that extend the life. Food seems to be another source of damage since many of the genes involved in the reduction of ageing are also related to the answer of the organism to changes in the levels of nutrients (Masoro, 2000; Partridge and Gems, 2002).

2. THEORIES OF AGEING

Among the huge number of theories that have been proposed, only a few of them are able to explain the fall of homeostasis at the end of the life. Variability in the extent of the life between groups or the life extension through mutations or experimental approaches must be considered (Sohal et al., 2002). Among these theories, the most accepted does Harman (1956) propose the free radical theory of ageing. This theory enunciates that normal ageing is the result of the stochastic damage to tissues mediated by free radicals. Later, Harman focused his theory to the mitochondria as the main source and target of free radicals (Harman, 1972). In 1980, Miquel et al. proposed the mitochondrial theory of ageing (progressive damage to mitochondrial DNA by reactive oxygen species (ROS). Since nowadays it is well known that many ROS are not free radicals, the above-mentioned theory are known as the oxidative stress theory of ageing. In addition, there are many other theories among which it deserves mention the genetic theories of ageing (Finch and Ruvkun, 2001). Briefly, this group of theories proposes that ageing is the extension (as a sequence of events codified by the genome) of the processes of development and differentiation. These theories are based in the assumption that ageing would be an ultimate consequence of the expression of genes selected by evolution because its success in reproduction.

3. THE ROLE OF MITOCHONDRIA IN AGEING

Progressive loss of mitochondrial functionality is one of the common events associated to ageing. This fact leads to consider these organelles as the biological clock of ageing (Salvioli et al., 2001). In fact, mitochondria have been proposed as the link between the age-dependent accumulation of oxidative damage produced by ROS and the physiological alterations associated to ageing (Van Remmen and Richardson, 2001). In that sense, several experimental evidences suggest that mitochondria are one of the main targets of the ageing process. Among those evidences, we can found (Salvioli et al., 2001):

- Accumulation of deletions and punctual mutations at the mitochondrial DNA (mtDNA) and decrease in the mtDNA copy number in some tissues.
- Age-dependent decline in the activity of some enzymes at the mitochondrial electron transport chain (mtETC).
- Increase in the production of free radicals, probably because of the previously described alterations.
- Alterations in the morphology of mitochondria and collapse of the mitochondrial membrane potential ($\psi_{mt}$).

Variations in the functionality of mitochondria may be important to achieve an adequate or inadequate ageing. At least, that can be ascertained from the study of specific population groups around the world. For example, an inherited variation of an mtDNA germ line (halogroup J) has been associated to a more adequate ageing process and to an extended life in the Italian population (De Benedictis et al., 2000). On other way, it has been discovered in Japan three mutations associated to an mtDNA germ line found with a high frequency in centenarians from this part of the world (Tanaka et al., 1998).

Another interesting question is the role of mitochondria as a very important element from the point of view of cell signal transduction. Thus, mitochondria may also be considered as an element of control for the nuclear gene expression. In this sense, a number of adaptation or regulation proteins have been found at the mitochondrial level or are translocated to the mitochondria to cop with this role. That is what happens with Nur77/TR3, p53, PKC, JNK/SAPK, some caspasers and several members of the bcl2 family like Bid, Bax or Bim (Finkel and Holbrook, 2000).

In relation to the role of mitochondria in the ageing process, the control of apoptosis is important.
This control is frequently lost in aged cells, which in addition are more prone to suffer from oxidative stress. ROS decrease $\psi_{mt}$, allowing the opening of the transition pore and the subsequent escape to the outside of calcium and other substrates. This sequence of reactions leads to apoptosis in lymphocytes, liver and the brain of aged mice (Watson et al., 2000).

4. MITOCHONDRIA AS THE CELLULAR SOURCE OF ENERGY AND OXIDATIVE STRESS

During the last years, it has been ascertained that although ROS are produced through a large number of pathways of the aerobic metabolism, the main source of these species is the mitochondria (Lenaz, 1998; Halliwell and Gutteridge, 1999; Cadenas and Davies, 2000; Sastre et al., 2000; Salvio et al., 2001; Van Remmen and Richardson, 2001). The inner mitochondrial membrane is very different from the rest of biological membranes since its protein content goes beyond 80% (most of biological membranes do not exceed of 50%) (Quiles, 1995). Because of its importance and significance in the context of oxidative stress, it deserves to be mentioned the protein complexes integrated in the mitochondrial electron transport chain (mtETC). In aerobic organisms, the mtETC produces the energy needed for the life support. Basically, food are oxidized through the loss of electrons that are accepted by electronic carriers like the nicotinamide adenine dinucleotide mononucleotide, FMN, and flavin adenine dinucleotide, FAD. The reduced nicotinamide adenine dinucleotide (NAD$^+$) and flavines (flavin mononucleotide, FMN, and flavin adenine dinucleotide, FAD). The reduced nicotinamide adenine dinucleotide (NADH) and the reduced flavines (FMNH$_2$ and FADH$_2$) are oxidized again by oxygen producing great amounts of ATP. Oxidation is carried out by small jumps in which energy is gradually liberated (Lenaz, 1998). Five lipoprotein complexes (Quiles, 1995; Lenaz, 1998; Cadenas and Davies, 2000) mainly compose mtETC:

1) Complex I, NADH dehydrogenase complex.
2) Complex II, succinate dehydrogenase.
3) Complex III, bc$_2$ complex.
4) Complex IV, cytochrome c oxidase (COX).
5) Complex V, ATPase.

The mtETC fraction that metabolises oxygen is Complex IV. This enzyme uses four molecules of reduced cytochrome c to remove one electron to each one and to give them to an oxygen moleule. This tetra-electronic reduction of the oxygen is not suitable in a single step but it must be done electron by electron. Because this gradual reduction, the protein complex must be sure that partially oxidised oxygen, highly toxic, will not leak to the medium before to be transformed to water.

Superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$) are respectively the product of the monovalent and bivalent reduction of oxygen. Both species are usually produced during aerobic metabolism, mainly at the mitochondrial level (Cadenas and Davies, 2000). It has been estimated that a 1-5% of the consumed oxygen through the mitochondria is not fully reduced to water. In turn, this small oxygen percentage is transformed to $O_2^-$ which spontaneously or as the result of the action of superoxide dismutase enzymes is transformed to $H_2O_2$. Although COX is the enzyme involved in the oxygen reduction, it almost does not generate free radicals. In opposition, the two main sites of free radicals production at the mtETC are complex I and complex II (Cadenas et al., 1977; Ksenzenko et al., 1983; Shimomura et al., 1985; Cross and Jones, 1991). What happens is that during the pass from one complex to the other, some electrons escape and directly join to surrounding oxygen resulting in the generation of $O_2^-$ and $H_2O_2$. Moreover, at the external mitochondrial membrane there is an additional source of ROS. This source comes from the desamination process of biogenic amines by monoamine oxidases, which through a bi-electronic reduction produce $H_2O_2$ from $O_2$ (Hauptman et al., 1996). The physiological level of ROS production at the mtETC depends on the metabolic state of the mitochondria. Thus, the state of mitochondrial rest (state 4), characterized by a low respiration level and no ADP availability, is associated to a high rate of ROS production, probably because of the high degree of reduction of the chain components. The active mitochondrial state (state 3), characterized by high oxygen expending and elevated ADP availability, shows a relatively low ROS production. In the state of anoxia (state 5), distinguished by a limitation in the oxygen delivery and absence of respiration, no ROS production is observed (Cadenas and Davies, 2000).

Biological membranes are overall very sensitive to oxidative stress because the presence of double bond carbon-carbon in the lipid tails of its phospholipids (Montine et al., 2002). Oxidative damage to membrane lipids may be directly generated trough initiation by ROS as hydroxyl radicals or the superoxide anion, or indirectly by some products of the same lipid peroxidation like some highly reactive aldehydes that maximize the phenomenon (Esterbauer et al., 1991). Irrespective of the way, oxidative damage of membrane lipids leads to its alteration and to changes in membrane fluidity and because of all these changes to alterations in membrane function (Halliwell and Gutteridge, 1999). Moreover, there is a particular mitochondrial lipid called cardiolipin. This is a highly unsaturated lipid and consequently highly prone to oxidation. Cardiolipin oxidation is extremely important to mitochondria since it is involved in the function of mtETC proteins like COX or the adenine nucleotide transporter (ANT) (Paradies et al., 1998).
Since lipids and proteins are physically very close, oxidative damage to mitochondrial proteins, as result of direct oxidative stress or because of lipid peroxidation, may lead to cross-linking, degradation and loss of function of such proteins. Several membrane proteins like ATPase, ANT, COX, ..., are easily inactivated by oxidative stress. Moreover, protein oxidation leads to the opening of the permeability transition pore, a key step in the process of apoptosis. In summary, mtETC protein alteration has as a direct consequence the loss of mitochondrial functionality and indirectly a rise in the ROS production (Lippe et al., 1991; Forsmark-Andree et al., 1997).

Mitochondria have an own genome, which is different in structure and organization to the nuclear genome. It is composed of a variable number of copies of identical circular double-strand DNA (up to ten copies). It is localized in the mitochondrial matrix, near to specific areas of the inner mitochondrial membrane (e.g. close to the main source of ROS). It has a small size (16.5 Kb) and codifies for 13 mitochondrial proteins: seven subunits from complex I, one protein from complex III, three proteins from complex IV, two from complex V (ATPase), twenty-two trRNA and two rRNA (Lenaz, 1998; Cadenas and Davies, 2000; Van Remmen and Richardson, 2001). In opposition to nuclear DNA, mitochondrial DNA is not protected by histones and it has been traditionally considered of a high susceptibility to be oxidatively attacked (Richter et al., 1988). For a long time, it was considered that mitochondrial did not have a system to repair damaged DNA. Nowadays, it has been reported the existence of such a system, although it is not yet well known (Bohr and Anson, 1999).

There are many reports suggesting that oxidative damage to mtDNA is more important from the point of view of ageing that the exerted to lipids and proteins. This fact is due to the ability of mtDNA to be spread because the division capacity of mitochondria and cells, which allows amplifying the physiological consequences of the exerted damage. Furthermore, oxidative damage to mtDNA might be even more important than the damage to the nuclear DNA as the entire mitochondrial genome codifies for genes that are really expressed, while the nuclear genome contains a huge amount of nontranscribed sequences (Van Remmen and Richardson, 2001). Oxidative stress may affect mtDNA under several ways among those the most typical are the oxidative alteration to bases, the raise in the number of deletions and the occurrence of punctual mutations. To the moment, the most popular approach to study oxidative alterations to bases is through the analysis of 8-hydroxy-deoxyguanosine (8-OHdG) by HPLC attached to electrochemical detection. Using this procedure, several labs have reported higher levels of this biomarker at the mitochondrial level with respect to the values found in the nucleus during ageing (Chung et al., 1992; Agarwal and Sohal, 1994). Concerning DNA deletions, it has been described that there is a raise in the frequency of these events with ageing in a wide variety of post-mitotic tissues from several species, including humans, monkeys, rodents and nematodes (Yoneda et al., 1995). Moreover, the rise in the percentage of deletions has been directly correlated with oxidative damage. Among the most studied deletions, does exist one that has been called the "common deletion" because its frequency and that increases two to three times with ageing in some tissues like the brain (Cortopassi et al., 1992). Nonetheless, since the percentage in which the level of deletions increases does not exceeds from 2-3%, it is speculated about the actual physiological significance that this phenomenon could have from the point of view of ageing (Van Remmen and Richardson, 2001). mtDNA mutations are the base for an important number of human pathologies. That has opened a new and exciting field in the mitochondrial research. mtDNA has a maternal transmission, moreover there are many copies of the molecule in single cell (polyploidy) and it exists the chance that a mutation experiences different degrees of heteroplasmy. All these questions imply that a lesion is manifested only when around 80% of all the mtDNA in the cell become mutated (Lenaz, 1998; Michikawa et al., 1999).

5. OXIDATIVE STRESS AND ANTIOXIDANT DEFENCES IN AGEING

Several studies have report that the levels of different markers of oxidative stress rise with ageing, although some other studies did not found such increase (Halliwell and Gutteridge, 1999). Such a disparity in the results may be found in the nature of the biological sample or in the studied biomarker. Concerning the nature of the biological sample, many studies have been carried out using tissue homogenates or whole cells. In relation to that, it must be aware of the fact that most of the oxidative stress in the cell comes from mitochondria. According to that, a bad choice in the biological sample may lead to the hiddenness of existing differences (mtDNA represents a 5% of nuclear DNA). Furthermore, the net oxidative stress seems to be dependent of the sex of the animal, the specie, the studied tissue, the lipid profile of mitochondiral membranes, ... Once previous reservations have been done, enhanced levels of exhaled pentane and ethane related to age in old rats have been reported. In a similar way, higher levels of carbonyl radicals and 8-OHdG have been found in the brain and other tissues from rats, mice and humans (Sagai and Ichinose, 1980; Sohal and Dubey, 1994; Lee et al., 1997).
According to Halliwell and Gutteridge (1999), the steady state level of oxidative stress is the result of the balance between the levels of damage and the degree of repair or replacement of damaged molecules. According to that, in terms of balance, a net increase of oxidative stress during ageing may be found in terms of higher levels of damage as it or as the result of defects in the repair system. In that sense, it has been observed a positive correlation between efficiency of DNA repair system and the supplementation time ago. That link has been focused mainly at the conceptually, the term “oxidative stress” apart to several cell lines to degrade abnormal proteins and to repair DNA seems to diminish with ageing. Thus, antioxidant dosage must be carefully adjusted in relation to cell signalling. According to that, the antioxidant capacity should be extended to the complex, and not yet well understood, framework of the damage repairing systems (Bohr and Anson, 1999).

In relation to the response of antioxidant defences, it seems that overall protection does not decline with ageing (Kellog and Fridovich, 1976; Quiles et al., 2004), although several exceptions have been described.

6. NUTRITION AND AGEING

Nutrition has been related to ageing since long time ago. That link has been focused mainly at the level of caloric restriction and the supplementation with antioxidant molecules. McCay et al. (1989) firstly described the role of caloric restriction, e.g. the limitation in the food intake, in 1935. Since then, it has been described how caloric restriction enhances mean life span in a wide range of species, decreasing in rodents the speed to what some related-diseases develop (Masoro, 2000; Finkel and Holbrook, 2000). These effects are carried out by a reduction in the oxidative stress level. That is supported, among other, by the fact that calorically restricted animals experience less oxidative stress than their counterparts fed ad libitum (Masoro, 2000; Finkel and Holbrook, 2000). In addition, caloric restriction prevents from many of the changes found at the level of gene expression during ageing (e.g. increase in the expression of heat shock proteins). Caloric restriction might be a powerful therapeutic tool to fight against ageing since, a priori, it fulfill with the required needs of effectiveness against oxidative stress and ageing (Roth et al., 1999). Nevertheless, the possible use of caloric restriction as anti-ageing therapy in humans results in such practical and ethical difficulties that make almost impossible its actual development (Finkel and Holbrook, 2000).

As it has been stated in previous paragraphs, oxidative stress plays a very important role in the global process of ageing. Thus, nutritional supplementation with molecules or substances endowed with antioxidant capacity should be useful as a possible anti-ageing therapy. Miquel and Economos (1979) performed some of the first studies in this field. These authors studied the capacity of thiazolidine carboxylate to enhance vitality and enlarge mean lifespan in mice. Later, Furukawa et al. (1987) showed the role of glutathione in the protection against the immune function decline associated to ageing. Many other antioxidants have been tested in relation to ageing, showing more or less positive results. Among these antioxidants it deserve to be mentioned vitamin E, vitamin C, coenzyme Q, herbal extracts rich in polyphenols and flavonoids, and others (Halliwell and Gutteridge, 1999; Huertas et al., 1999; Watson et al., 2000). Although results found with these antioxidants have been successful in relation to the attenuation of the age-related oxidative stress, they had low or no success concerning extension of the lifespan. Perhaps, to be more successful with antioxidants-related therapy, we should deepen into the pharmacological properties of the studied molecules, particularly in relation to aspects like absorption, tissue distribution and metabolism. Furthermore, it should be remember the role of ROS in relation to cell signalling. According to that, the antioxidant dosage must be carefully adjusted in order to avoid changes in the redox state that could alter the cell function. Theses problems are being amended using a new generation of synthetic antioxidant substances, mimetics of the superoxide dismutase and catalase enzymes. These substances are being assayed with some success, for example in relation to the extension of longevity in mice and C. elegans (Melov et al., 1998; Rong et al., 1999; Melov et al., 2000).

7. OLIVE OIL, DIETARY FATTY ACIDS AND OXIDATIVE STRESS. A NEW APPROACH TO THE STUDY OF AGEING

Dietary fat type determines several biochemical parameters at the mitochondrial membrane level (Mataix et al., 1998; Quiles et al., 1999a). The importance of fatty acids resides in the fact that mitochondrial membrane (as the rest of the biological membranes) adapts its lipid composition to dietary fat (Huertas et al., 1991a; Quiles et al., 1999c; Ochoa-Herrera et al., 2001). Thus, if a person mainly eats animal fat, its biological membranes will be richer in saturated fat than a person mainly fed on vegetable oils. On other hand, adaptations of electron transport system in relation to dietary fat type have been widely reported (Battino et al., 2002a; Huertas et al., 1991b; Quiles et al., 2001). Moreover, oxidative stress is related to biological membrane composition. In that sense, a polyunsaturated fat source (e.g. sunflower oil) will lead to membranes more prone to oxidation than a...
saturated (animal fat) or a monounsaturated (e.g. olive oil) source. That has been widely demonstrated under a wide range of physiological and pathological situations using both animal models and humans (Quiles et al., 1999b, 2002b; Ramirez-Tortosa et al., 1999; Battino et al., 2002b; Ochoa et al., 2002).

According to the above-mentioned premises, dietary fat type affects mitochondrial structure and function as well as their susceptibility to experience oxidative stress. In this way, if we build “customized” biological membranes according to a particular dietary fat type, we would be able to modify in a positive form the way and degree in which different organs reach ageing. This work hypothesis represents a new approach to the study of ageing from the point of view of nutrition and it could have important repercussion in the study of the ageing phenomenon. Moreover, it might help us to understand the existing differences concerning ageing between different populations with a similar socio-economic level but with marked differences in their dietary habits. Something similar has been already demonstrated in relation to pathologies like cancer and cardiovascular disease. Thus, populations like the Mediterranean, with a fat intake different in type (or even higher in quantity), mainly olive oil, present lower figures for these pathologies than those found in northern Europe or the United Stated (where most consumed fats are saturated or highly polyunsaturated seed oils) (Mataix, 2001). Nevertheless, it is obvious that other factors, apart from dietary fat, are responsible for differences in the above-mentioned pathologies. However, the role of dietary fat (and olive oil in particular) seems to have enough importance to be carefully studied in relation to ageing.

Results found in our laboratory using virgin olive oil or sunflower oil as dietary fat in relation to mitochondrial ageing suggest the following mechanism and conclusions: Ageing, understood as an endogenous and progressive phenomenon (Barja, 2002), leads throughout lifespan to different disturbances in mitochondria and their components like mtDNA (Sohal and Dubey, 1994; Lee et al., 1997; Michikawa et al., 1999). These disturbances (which have a high oxidative component) declines mitochondrial structure and function. Depending on the capacity of the affected tissue to repair the damage or to replace the altered cell, tissue function will be affected in a higher or lower extent (Quiles et al., 2002). In that way, tissues with the ability to regenerate their cells, like liver, seems to be able to buffer at least in part the damage, as it has been suggested by the lack of changes in the mitochondrial function in terms of cytochrome c oxidase activity (Quiles et al., 2002). However, a loss in function is found in postmitotic tissues like skeletal muscle, heart or brain. These tissues have not the chance to replace damaged cells and probably they have a less effective repairing system [differences between liver and heart concerning repair mechanisms for mtDNA damage have been already reported (Souza-Pinto et al., 1999)]. This loss in function is reflected in the deep fall in cytochrome c oxidase activity, which leads to the uncoupling of the mETC, with the further bioenergetic inefficacy and the raise in the ROS production (Quiles et al., 2002; Ochoa et al., 2003). Mitochondria from postmitotic tissues try to buffer the unfavourable situation by the increase in some elements of the mETC like cytochrome b or polyunsaturated fatty acids (PUFA). The increase in polyunsaturation tries to enhance membrane fluidity and the cytochrome c oxidase activity by the presence of a more polyunsaturated cardiolipin (Huertas et al., 1991b; Quiles et al., 2001). However, both actions lead to a raise in the ROS production. The role of dietary fat in this mechanism could reside in the building of an environment more or less prone to the generation and propagation of ROS, especially when as the result of events like ageing, failures in the mETC start to appear.

Moreover, dietary fat could modulate the phenomenon through variations in the antioxidant system and overall upgrade or attenuate the process. Thus, as postmitotic tissues are the most affected by ageing, diet should be particularly important at these tissues. Therefore, when dietary fat is dispensed as virgin olive oil versus sunflower oil, a better general state at the mitochondrial function level is found, with a lower ROS production and finally with a delay in the occurrence of the ageing phenotype. That leads to conclude that from the point of view of ageing, the intake of virgin olive oil presents important advantages when comparing with other dietary fat sources.

In summary, results previously described open a new and exciting way to investigate the mechanisms involved in the benefits of (extra virgin) olive oil in relation to ageing. In that sense, new studies are being developed to investigate aspects like the possible modifications of the mtDNA repair systems or the changes in the nuclear and mitochondrial gene expression profile after the intake of virgin olive oil and their consequences on the ageing process.

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