Chapter 10
Coenzyme Q, mtDNA and Mitochondrial Dysfunction During Aging

José L. Quiles, Alfonso Varela-López, María D. Navarro-Hortal, and Maurizio Battino

Abstract The main sources of reactive oxygen species (ROS) in cells are mitochondria, whose components would be primary targets of ROS. Both facts are responsible for the key role of these organelles in aging according to the "mitochondrial theory of aging". Oxidative damage to mitochondrial DNA (mtDNA) is especially important since it would have the longest-term consequences impairing mitochondrial function. This would lead to a decrease in ATP production, but also to an increased ROS generation. In turn, CoQ, which acts as an electron carrier in mitochondria, is an essential factor for cell bioenergetics and an equilibrated CoQ pool is expected to perform a better electron flow adaptation. Moreover, it is a lipid-soluble antioxidant and efficiently prevents oxidation of DNA along with other macromolecules. Other interesting attributed roles include interaction with cell signaling cascades, anti-inflammatory activities and interference with programmed cell death. Due to this pleiotropic effect, most of interventions with CoQ have been focused on multiple processes related to mitochondria. In this sense, its effects have been investigated in mitochondrial diseases and pathological conditions related with aging whose patients have shown a higher frequency of mtDNA alterations. In addition, dietary CoQ also has been tested in combination with different diets rich in particular type of fatty acids due to the role of these in biological membranes and oxidative stress, as well as aging. This chapter aims to review the effect of CoQ on aging and mitochondrial dysfunction, with especial interest in their actions on mtDNA or the consequences of mtDNA alterations.

Keywords Diet · Mitochondrial diseases · mtDNA mutation · Oxidative stress · ROS · Ubiquinone

J. L. Quiles (✉) · A. Varela-López · M. D. Navarro-Hortal
Department of Physiology, Institute of Nutrition and Food Technology "Jose Mataix Verdú", University of Granada, Biomedical Research Center, Granada, Spain
e-mail: jlquiles@ugr.es

M. Battino
Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche (DISCO)-Sez. Biochimica, Facoltà di Medicina, Università Politecnica delle Marche, Ancona, Italy

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10.1 Introduction

There is a growing body of evidences indicating that mitochondria have a key role in aging phenomenon particularly in organs or tissues with an important dependence of aerobic metabolism. A major fact explaining this possible role is that mitochondria are the main source of reactive oxygen species (ROS) in most cells. An impairment of mitochondrial function led to lower production of ATP, but other important downstream consequences also should be considered. Among other, the increase in the generation of reactive oxygen species is usually considered as the most relevant.

In turn, oxidative stress also seems a major factor influencing mitochondria "health" since primary target of ROS would be mitochondrial components. Oxidative damage to mitochondrial DNA (mtDNA) would be especially important since it would have the longest-term consequences in mitochondrial function. For these reason, there are several theories of aging that have suggested a key role of mitochondria and in particular of mtDNA in this phenomenon.

This chapter aims to review the effect of coenzyme Q (CoQ) on aging and mitochondrial dysfunction, with especial interest in their actions on mtDNA or mtDNA mutations consequences. Additionally, to improve the understanding of implications in aging of oxidative damage to mtDNA, this chapter provides an approximation to mtDNA processes as well as an analysis of mechanisms that try to explaining their relationship with aging.

10.2 Mitochondrial DNA Features

Unlike the rest of organelles (except chloroplasts in vegetal cells), mitochondria had their own extrachromosomal DNA molecules. mtDNA presents structural and functional features very different respect than nuclear DNA (nDNA). For this reason, a preliminary approach to these unique features is needed to properly understand the relationship of mtDNA with mitochondrial functionality and aging.

MtDNA is a circular double-stranded molecule located in the mitochondrial matrix whose size ranged from 16,000 to 18,000 base pairs (bp) in vertebrates. Namely, human mtDNA size is 16569 bp and its sequence was determined in 1981 (Anderson et al. 1981). To differentiate the two mtDNA strands, it has been established the terms "heavy strand" (H-strand) and the "light strand" (L-strand) according to their GCT content. This is due to their behavior when strands are separated on denaturing cesium chloride gradients. MtDNA has no histones but rather packaged into nucleoids. These consist in stable protein-mtDNA macrocomplexes primarily associated to inner mitochondrial membrane (Wang and Bogenhagen 2006; Holt et al. 2007). Each nucleoid has an average diameter of 100 nm (Kukat et al. 2011) and they may be exchanged between mitochondria (Wang and Bogenhagen 2006; Holt et al. 2007).

Mitochondrial genome encodes 13 polypetides, two rRNAs and 22 tRNAs (Anderson et al. 1981). Encoded polypetides are subunits of mitochondrial electron transport chain (mtETC) complexes whereas rRNAs and tRNAs are required for the intramitochondrial translation of the protein-coding units. In contrast to nuclear, mitochondrial genome does not contain introns within mtDNA coding region and almost no noncoding nucleotides exist between genes. In vertebrates, an exception is a noncoding region closely associated to origin of H-strand DNA replication (O_H) that contains the transcription promotors (Clayton 1982, 1991). In most of cases (some species present two), each strand contain only a promoter. The transcription processes initiated from each of them yield two large polycistronic transcripts that are processed later to generate mature tRNAs, rRNAs, and mRNA by precise endonucleolytic cleavages. In most cases, such cleavages occur, immediately before and after a tRNA sequence. Depending on which strand acts as the template for transcription, promoters are designated as light-strand promoter (LSP) or heavy-strand promoter (HSP) (Ojala et al. 1981; Attardi and Schatz 1988; Clayton 1992). In addition to 13 mtDNA encoded subunits, there are other approximately 70 components of mitochondrial respiratory chain and other proteins that participate in mitochondrial metabolism and maintenance, which are encoded in the nuclear genome and require specialized import systems to be imported to mitochondria (Mokranjac and Neupert 2005).

Several molecules (usually from two to ten) of mtDNA coexist in a mitochondrion and there are mitochondria in every cell. Therefore, a cell possesses hundreds of copies of mtDNA, a condition termed as polyplasmy. When all mtDNA in a cell present the same sequence, the condition is known as homoplasmy. In contrast, heteroplasmy occurs when two or more different molecules of mtDNA can be also present in a cell or organism.

Because of zygote does not receive mitochondria from sperm in mammals or possible transmitted mitochondria are selected against during replication, all mitochondria are inherited from the mother. Then, mitochondria divide and proliferate during development, but also in adult life increasing mitochondrial mass. During this process known as mitochondrial biogenesis synthesis of new mitochondrial proteins, but also mtDNA, is required. It is known that biogenesis is under the control of nuclear factors, although the exact mechanism has not been fully unraveled yet.

10.2.1 Replication of Mitochondrial DNA

It is assumed that mtDNA replication is not necessary linked to the cell cycle (Clayton 1982) and mtDNA is continuously turned over. Thus, mitochondrial and nuclear genomes would be independently replicated (Bogenhagen and Clayton 1977). For this reason, mtDNA is also replicated in postmitotic cells, which has been also evidenced (Pohjoismäki et al. 2009). Notwithstanding, there is a increasing number of publications reporting some relationship between mitochondrial

function and cell cycle (Arakaki et al. 2006; Owusu-Ansah et al. 2008; Mitra et al. 2009), which suggests a possible connection between mtDNA replication and the cell cycle.

The exact mechanism of human mtDNA replication is not completely known yet. The two most important replication models proposed until now are: the strand-asynchronous method that is the most traditional and the leading-lagging strand model (Holt et al. 2000; Fish et al. 2004). In both, DNA replication is initiating at O_H that is located downstream of the LSP in the D-loop region. In the first one, subsequent elongation of a nascent newly-synthesized H-strand leads to the parental H-strand displacement from the H-strand. Because of the origin of L-strand DNA replication ($O_{L)}$ is located approximately two thirds the genomic distance away from O_H on the mtDNA molecule, L-strand synthesis, that proceeds in the opposite direction, will not be initiated until almost two-thirds of new H-strand have been synthesized and O_L is exposed (Clayton 1992). Here, the adoption of a particular configuration by the H-strand allows a mitochondrial DNA primase initiate L-strand DNA synthesis (Wong and Clayton 1985a, b). In the second model, L-strand synthesis starts in a coordinately way shortly after replication in form of short Okazaki fragments that will be joined then (Yasukawa et al. 2006).

In any of the models, after DNA strand synthesis, the two daughter molecules will be separated, RNA primers removed, and remaining DNA gaps will be filled and ligated. An additional step introducing superhelical turns into the closed molecule also occurs (Shadel y Clayton 1997). The existence of a model does not necessarily exclude the other and their occurrence may depend on cell type. It has been suggested that cells requiring rapid mtDNA synthesis present a strand-displacement mechanism whereas the leading-lagging strand one would be more prevalent in cells which are in a steady-state (Holt et al. 2000; Fish et al. 2004; Jacobs et al. 2006; Tuppen et al. 2010).

Regardless of the model, various nuclear DNA (nDNA)-encoded proteins are needed to form the mitochondrial replisome and accomplish mtDNA replication. These include a 5′–3′ DNA helicase named Twinkle, some mitochondrial SSB proteins (mt-SBB) and the polymerase γ (Polγ) that contains two subunits, one catalytic with 5′-3′ exonuclease activity (PolγA) and other processivity (PolγB) (Korhonen et al. 2004).

10.2.2 Mutations and Mitochondrial DNA

Molecular defects in mtDNA have a significant role in human disease and aging and they have been found in each type of mitochondrial gene. MtDNA mutations range from single base changes in the genome (point mutations) up to large rearrangements (deletions and duplications). In turn, these changes in mtDNA sequence can be maternally inherited or somatic (i.e. created *in situ*). In general, deletions and duplications are most often sporadic or somatic whereas maternally inherited alterations are commonly point mutations (Leonard y Schapira 2000).

Alterations in mtDNA sequence have been strongly associated with deleterious effects on organisms. There are at least two reasons explaining this relationship. On the one hand mtDNA and has no introns, so that a random mutation will usually strike a coding DNA sequence. On the other hand, estimations indicate a 10–20 fold higher mutation frequency in human mtDNA than in nDNA (Brown et al. 1979). This higher rate could be due to the combination of two factors. On the one hand, maybe the number of systems of DNA repair is insufficient for all the damage that occurs, although there are a growing number of reports indicating that in mitochondria there are more enzyme activities for repair of damaged nucleotides of which it was believed at first. On the other hand, it seems that mtDNA has an increased susceptibility to mutation (Shadel and Clayton 1997). Several differential features are responsible for this susceptibility to mutation:

- Mitochondrial oxidative environment, mainly generated by free radicals generated at the electron transport chain, although there are other sources.
- The absence of protective histones, although mtDNA is packaged into protein-mtDNA aggregates termed nucleoids (Kukat et al. 2011) that are believed to protect mtDNA from chemical damage in some degree (Lagouge and Larsson 2013).
- Failure of proof-reading by mtDNA polymerases during mtDNA replication that usually also occurs although cell does not divide.
- Lack of recombination as consequence of maternal heritage that allows sequential accumulations of mutations through maternal lineages.

Mutations in mtDNA have received great interest because it is known that specific mtDNA mutations found in humans are likely causative in different diseases. Moreover, common neurodegenerative disorders and others disease often associated with aging also have been associated with mtDNA sequence alterations (Larsson and Clayton 1995). Because of cells present polyplasmy, normal and mutant mtDNA can coexist within the same cell. Actually, the existence of either completely normal or completely mutant mtDNA is rare. In this context, heteroplasmy is particularly important since it can allow an otherwise lethal mutation to persist. This is due to a certain minimal amount or threshold level is required to have deleterious effects in cell (Larsson 2010). In that sense, there are selection pressures at the molecular and cellular levels, as well as at the level of the organism itself. The proportion of mutant mitochondrial DNA required for the occurrence of a deleterious phenotype, known as the threshold effect, varies among persons, among organ systems, and within a given tissue.

After mtDNA sequence alterations are produced, several processes can modify their frequencies in the cell. In this sense, it has been suggested that changes in the frequencies of different mtDNA molecules follows principles of population genetics but rather Mendelian laws. Both, *de novo* and inherited mutations in mtDNA, if there are present in heteroplasmy, are subject to mitotic segregation. Consequently, frequency of different mtDNA molecules can shift in daughter cells since they are randomly segregated during mitosis. Thus, mutated mtDNA can increase with possible deleterious effects or decrease to disappear, particularly in fast-dividing tissues (Tuppen et al. 2010). Anyway, mechanisms for mitotic segregation need to be

studied further. On the other hand, a mtDNA molecule may be replicated many times or not at all as a cell divide. If mtDNA molecules are selectively replicated, proportions of mutant and normal molecules in mother cells would be modified. In addition, it is important to note that replication of mtDNA also occurs in the absence of cell division. Thus, mtDNA is replicated also in postmitotic cells, so it can undergo similar types of segregation (Larsson 2010). It has been suggested that it is caused by random genetic drift, in conditions of relaxed mtDNA replication (Elson et al. 2001). Actually, expansion in postmitotic tissues, a preferential amplification of mtDNA mutations might occur termed clonal (Larsson et al. 1990; Weber et al. 1997).

In mammals, a rapid segregation in heteroplasmic mtDNA genotypes returning to homoplasmy in some descendants has been reported (Upholt and Dawid 1977; Olivo et al. 1983; Holt et al. 1989; Vilkki et al. 1990; Larsson et al. 1992; Blok et al. 1997; Brown 1997). The existence of a mtDNA bottleneck during development has been proposed to explain these observations (Tuppen et al. 2010). Different mechanisms by which this bottleneck is present have been hypothesized, but discussion about this topic remains. A relatively well-accepted hypothesis suggests that a marked reduction in mtDNA copy number would take place in the germ line leading to a genetic bottleneck during embryonic development (Jenuth et al. 1996; Cree et al. 2008). In contrast, other authors have suggested that, during oogenesis, there is a preferential replication of a particular mtDNA or a subgroup of them, but neither reduction of mtDNA copy number is produced in germ line (Cao et al. 2007). Other recently proposed explanation suggests that mtDNA subpopulation is selectively replicated during postnatal folliculogenesis, thus the mtDNA bottleneck would not occur during oogenesis. In single germ cells, mtDNA heteroplasmy and copy number vary throughout oogenesis (Wai et al. 2008), a finding that support that hypothesis. Anyway, more research is needed to clarify the mtDNA bottleneck exact nature (Tuppen et al. 2010).

10.2.3 Mitochondrial DNA Repair Systems

Although its importance is currently discussed (Richter et al. 1988; Hegler et al. 1993), oxidative damage occurs normally and can be elevated in cells and tissues (LeDoux et al. 1992; Mecocci et al. 1993, 1994; Driggers et al. 1993; Shigenaga et al. 1994). However, mitochondria have their own repair systems for damaged mtDNA that help to maintain mtDNA integrity, although their number seems to be more limited than in nucleus.

Base excision repair (BER) is one of the most studied mitochondrial mechanisms for mtDNA repair. In fact, intially it was though that short-patch BER was the unique pathway to repair mtDNA damage, especially oxidative damage (Stierum et al. 1999). This mechanism represents the main pathway for repairing oxidized modifications (Slupphaug et al. 2003), but it is also a primary pathway for alkylation and deamination-derived modifications repair (Dianov et al. 2001; Chan et al. 2006).

First step in BER is the cleaving the N-glycosidic bond leading to an abasic site. This reaction is catalyzed by different DNA glycosylases that are responsible to recognize modified bases and also present AP lyase activity to cleavage DNA backbone (Robertson et al. 2009). Among other, these include the uracil DNA glycosylase (UNG), the endonuclease III homolog (NTH1), and the 8-oxoguanine DNA glycosylase-1 (OGG1). OGG1 is particularly interesting since it is required for the recognition and cleavage of 8-oxoguanine (8-oxoG) from double-stranded DNA (Kuznetsov et al. 2005). In this step also participates the AP endonuclease (APE1) that cleaves on the immediate 5´ side of the apurinic/apyrimidinic (AP) site, leaving a 3´ hydroxyl and 5´-deoxyribose-5-phosphate (5´-dRP) residue (Masuda et al. 1998). Then, the resultant gap is filled with the correct nucleotide by the mitochondrial DNA polymerase -i.e. Polγ- (Ropp and Copeland 1996).

Here, it is possible to distinguish two BER pathways according to the number of nucleotides incorporated to the gap. When a single nucleotide is incorporated, the mechanism termed short-patch BER is relatively simple. In contrast, long-patch BER, which involves the incorporation of multiple nucleotides (commonly ranged from 2 to 7) is more complex and additional enzymatic activities are required (Robertson et al. 2009). Such enzymatic activities would deal with the exposure of the original DNA strand as a single-stranded overhang or a flap structure that is the main difficulty generated by the incorporation of several nucleotides (Xu et al. 2008). Finally, the nick generated is sealed by the mitochondrial DNA ligase, ligase III (Lakshmipathy and Campbell 1999a).

Other known nuclear DNA repair mechanisms has been proposed to exist in a mitochondrial version. It seem that the most clear additional mechanism is homologous recombination (LeDoux et al. 1992; Ling et al. 1995; Sage et al. 2010) that is the primary pathway to repair double-strand breaks. That plays a critical role in facilitating the progression of replication when advancing polymerase complex progress is blocked by the presence of a DNA lesion. There are also evidences for existence of mismatch repair (Mason et al. 2003) and non-homologous end-joining activities (Lakshmipathy and Campbell 1999b) at mitochondria but more research is needed to confirm them. Other hypothesized mechanisms especially useful to repair 8-oxoG have been nucleotide excision repair (Stevnsner et al. 2002) and translesion synthesis (Pinz et al. 1995; Graziewicz et al. 2004, 2007), although none enzyme activity related to them has been reported in mitochondria up to date.

10.3 Aging and Mitochondrial DNA

10.3.1 Aging and Mitochondrial DNA Mutations Relationship: A Conceptual Framework

Overall, different studies have indicated mutated mtDNA molecules accumulate with aging since elderly people has shown higher levels of somatic point mutations or deletions in mtDNA from different tissue types (Cortopassi and Arnheim 1990;

Simonetti et al. 1992; Laderman et al. 1996: Melov et al. 1999; Berneburg et al. 2004; Bender et al. 2006; Marín-García et al. 2006; Krishnan et al. 2008). This association between aging and mtDNA alterations also has been in found studies in rodents (Pikó et al. 1988; Quiles et al. 2006, 2010; Ochoa et al. 2011). However, discussion exists about what is the magnitude of such accumulation and its importance in aging. Still, rather low, the differences between young and old individuals in frequency of mtDNA mutations are statistically significant (Pikó et al. 1988). Additionally, an accumulation of multiple mtDNA deletions has also been reported in individuals with neurodegenerative diseases, such as Alzheimer and Parkinson's disease (Cortopassi et al. 1992; Coskun et al. 2004; Bender et al. 2006; Kraytsberg et al. 2006 Krishnan et al. 2008). Similarly, overall mtDNA heteroplasmy seem to increase with aging indicating that additional somatic mutations are continuously appearing during adult life (Pliss et al. 2011; Sondheimer et al. 2011; Diot et al. 2016). Most of these observations support the idea of mtDNA alterations and their subsequent accumulation during life are responsible or at least contribute to the senescent phenotype. Several proposed theories that related mitochondria and aging providing an explanation for this phenomenon.

As indicated above, mitochondrion is a major site of ROS production in the cell (especially at mtETC) which would makes mitochondria the prime targets for oxidative damage (Harman and others 1955; Miquel et al. 1980). This fact was taken into account by Harman and other authors (Harman and others 1955; Miquel et al. 1980) to considered mtDNA mutations to be the initiating, primary event in the aging process in their mitochondrial free radical theory of aging. According to that, a vicious cycle would be established whereby oxidative damage to mtDNA and other mitochondrial components leads to respiratory chain dysfunction, which in turn leads to increased generation of ROS, further facilitating respiratory chain components damage and thus creating a self-amplifying deterioration. The mitochondrial free radical theory of aging, thus, suggests the existence of a vicious cycle that results in an exponential increase in mtDNA mutations with time. Interestingly, Greaves et al. (2014), using next-generation sequencing, has reported that mtDNA mutation rate does not seem to increase with age (Greaves et al. 2014). This fact would be contradictory with the exponential increase in mtDNA mutations proposed by the mitochondrial free radical theory of aging.

More recently, new evidences have emerged that give more subtle roles beyond those as damaging agent to ROS. Actually, despite lifespan and ROS production is correlated, it has been shown that ROS are not directly responsible for aging (Sanz et al. 2010). However, it has been progressively appreciated that ROS also can function as signaling molecules, facilitating adaptation to stress in a wide variety of physiological situations (Sena et al. 2008). In this context, Hekimi and colleagues (2011) proposed the gradual ROS response hypothesis that suggests that "ROS generation is not a cause of aging, but rather represents a stress signal in response to age-dependent damage". Concerning mitochondria, when respiratory chain dysfunction coupled with moderate increases in ROS levels, these act as stress signal that activates protective quality control pathways improving mitochondria quality. These finding also have resulted in the mitohormesis hypothesis (Tapia 2006;

Ristow and Zarse 2010). Despite of the existence of protective quality control pathways, a continuous or strong dysfunction of the mitochondrial respiratory chain would lead to a substantial ROS accumulation. Consequently, protective and defense mechanisms against oxidative stress would be overwhelmed (Tapia 2006; Zelenka et al. 2015). Indeed, evidences suggest that mtDNA controls longevity (Sanz et al. 2010) which is consistent with this theory.

Lastly, possible link between mitochondria (and mtDNA) and other important event in aging process have been also proposed. In this sense, Ahmed et al. (2008) have suggested that telomerase protects mitochondria from mild oxidative stress. Other possible causes would include the repression of PGC-1 promoter by p53 activated as consequence of telomere dysfunction or TERT activity effect on mtDNA repair (Monickaraj et al 2012; Tyrka et al. 2015).

10.3.2 Generation and Accumulation of Mitochondrial DNA Mutations

Different mechanisms have been proposed to explain the accumulation of mtDNA mutations with aging. Oxidative damage to mtDNA is often assumed as main responsible for age-associated somatic mtDNA mutations generation, although there are other agents able to produce DNA lesions that also could accumulate that might be important under some conditions. If the amount of mtDNA (oxidative) damage overwhelm the mtDNA repair mechanism, a progressively accumulation of mtDNA alterations or mutations would occur with aging. The involvement of ROS in the creation of mtDNA mutations is central to the mitochondrial free radical theory of aging and it is supported by correlative data showing higher levels of somatic mtDNA mutations in older than in younger mammals including humans (Larsson 2010). It is known that DNA bases can suffer until 24 oxidative lesions different (Evans et al. 2004). There are also 13 additional major products of oxidative damage to the sugar moiety (Evans et al. 2004). In spite of this number, most of investigations has been focused on the guanine adduct 7,8-dihydro-8-oxo-deoxyguanosine (8-oxodG) (Evans et al. 2004) that is considered one of the most abundant oxidative lesions that accumulate in mtDNA over time. One consequence of 8-oxodG presence in DNA is the transversion with adenine during replication due to the mispairing of 8-oxoG, but the biological significance for the majority of the lesions remains unknow (Larsson 2010). It has been reported that accumulation of 8-oxoG in mtDNA occurs with age (Szczesny et al. 2003). Initially, it was thought that *in vivo* levels of 8-oxodG were very high (Richter et al. 1988), although then this finding was attributed to overestimation by methodological problems (Hamilton et al. 2001). More recently a sequencing study in mouse showed that mtDNA transversion mutations not increased with age, so oxidative damage may not be a major source of formation of mtDNA mutations (Ameur et al. 2011).

Although the exact steady state level of oxidative damage in mtDNA is variable among tissues and the importance is discussed in the literature (Richter et al. 1988; Hegler et al. 1993; Shadel and Clayton 1997), such damage occurs normally and can be elevated in cells and tissues under certain conditions. These include exposure to certain chemical agents (Driggers et al. 1993) and antiviral drugs (Lewis and Dalakas 1995), UV radiations (Berneburg et al. 2004; Krishnan et al. 2008; Birket y Birch-Machin 2007) or pathologies (Mecocci et al. 1994). In this context, dietary conditions could results especially interesting for mtDNA mutation implications in aging since certain nutritional conditions may be maintained over life. Recent experimental studies indicate that reduction in the degree of unsaturation of fatty acids in the diet induces less oxidative damage and alterations in mitochondrial DNA (mtDNA) in different tissues including liver (Quiles et al. 2006), brain (Ochoa et al. 2011) and heart (Quiles et al. 2010).

An alternative source of mtDNA mutations is pol γ that would produce somatic mtDNA mutations by slipped mispairing during mtDNA replication. Namely, these replication errors have suggested being an important mechanism for formation of mtDNA deletions (Madsen et al. 1993). In human mtDNA deletions that mainly occur between O_H and O_L, are typically flanked by short direct repeated sequences (Mita et al. 1990; Samuels et al. 2004; Bua et al. 2006), which supports this hypothesis. Moreover, an *in vitro* analysis of the mutations generated by wild-type Pol γ showed a good concordance with those observed *in vivo* in human, including a paucity of G:C to T:A transversions (Zheng et al. 2006). This hypothesis is also supported by mathematical modeling (Cortopassi and Arnheim 1990). Still, the fact of true turnover rate of mtDNA in mammalian tissues is largely unknown complicates studies in this area (Larsson 2010).

However, most important evidences in favor of this mechanism come from studies using a well-established knock-in murine model (Trifunovic et al. 2004; Kujoth et al. 2005). This has homozygous genotype for a mutated version of PolγA with increased proofreading activity, so it provides a critical test of the replication error hypothesis. Expression of the proof-reading deficient PolγA leads to a rapid accumulation of mtDNA point mutations and deletion during embryogenesis, which are clearly present in midgestation (Trifunovic et al. 2004). Moreover, in adult life, accumulation of mtDNA mutations goes on in a linear manner leading to the progressive and random accumulation of mtDNA point mutations during mitochondrial biogenesis (Trifunovic et al. 2004). Because of this amount of mtDNA mutations, it has been generally named as Polγ mutator mouse. Most of the mutations generated in mtDNA mutator mice are transitions (Trifunovic et al. 2004) and their pattern after germ line transmission resembles the mutation spectra found in natural populations of mice and humans (Stewart et al. 2008a, b). Because of mitochondrial free radical theory of aging predicts an exponential increase in the mutation burden throughout life; findings from these models are in contradiction with it. Interestingly, accumulation of mtDNA mutations has no major increase in oxidative damage in many different tissues in adults (Trifunovic et al. 2005).

Most researchers consider replication to be the most likely mechanism of deletion formation (Lloret et al. 2009; Lagouge and Larsson 2013), but Krishnan et al.

(2008) proposed that "mtDNA deletions arise during the repair of damaged mtDNA". Although they remain unclear, some mechanisms have been proposed to explain the impairment or restriction of repair machinery efficiency with aging. A possibility would be the age-associated decline in import capacity of the mitochondria. Accumulation into the mitochondrial intermembrane space and importation failure inside the mitochondrial matrix of an unprocessed form of DNA glycosylase OGG1 that is involved in BER of 8-oxoG has been proposed to occur with aging (Szczesny et al. 2003). This would explain why 8-oxoG is so abundant among oxidative lesions that accumulate in mtDNA. In fact, mice lacking this enzyme have increased levels of 8-oxodG in mtDNA (de Souza-Pinto et al. 2001). In turn, mispairing of 8-oxoG during replications would extend the mutation

Several processes cooperate to maintain mitochondrial quality, among which highlights mitophagy that is the only mechanism known to turn over whole mitochondrial genomes (Kim et al. 2012; Diot et al. 2016). As it is expected, to keep the pool of mitochondria healthy, replacement by biogenesis is needed that must be adequately coordinated with mitophagy. Although mtDNA turnover in differentiated tissues is not well defined, if this results affected by aging, accumulation of mutant mtDNA can occur. In this sense, it has been reported a decline of mitophagy with aging (Diot et al. 2015) thus disadvantages both the turnover of dysfunctional mitochondria and the production of fresh mitochondria. Interestingly, Greaves et al. (2014), using next-generation sequencing, have shown that mtDNA mutation rate could not increase with age, which enhances the importance of autophagy decline in mutations accumulation.

Along with the aforementioned mechanisms, changes in mitochondrial dynamics also are very important, as well as affecting fusion and fission of membranes, modulate mitochondrial turnover. Fission disrupted mitochondria segregation (Katajisto et al. 2015) whereas fusion would mix content of different mitochondria including mtDNA molecules (Chan 2012; Tam et al. 2013). When the frequency of fusion/fission cycles is reduced, mtDNA mutations tend to accumulate and there is less mtDNA mixing (Diot et al. 2016). In support of these mechanisms importance, a mathematical model by Tam et al. (2014) suggests that a combination of rapid mitochondrial fission, fusion and mitophagy can extend lifespan because mitochondrial function maintenance would be achieved.

10.3.3 The Impact of Mitochondrial DNA Alterations on Mitochondrial Function and Aging

Independently of the actual cause of a given mutation, it is possible to suppose at least some of the consequences of changes in mitochondrial DNA sequence. Most mtDNA sequence alterations are neutral polymorphisms (Ingman et al. 2000), but when this not occurs, their magnitude could be different depending on gene affected. Point mutations can affect to protein, tRNA, or rRNA genes within mtDNA. Phenotypical consequence of mutations in a protein-coding gene would

be a functional alteration of a particular complex of mitochondrial respiratory chain to which the corresponding protein belongs (Tuppen et al. 2010). In turn, mutations in genes encoding for mt-tRNAs might impair overall translation of mtDNA by reducing functional mt-tRNAs availability (Tuppen et al. 2010). Regarding mtDNA rearrangements, it has been reported that most of them are large-scale deletions ranged from 1.3 to 8 kb that span several genes (Schon et al. 1989).

It is expected that accumulation of somatic mtDNA mutations would lead to mitochondria with respiratory chain deficiencies. Notwithstanding, an important feature of mtDNA further must be considered to understand the consequences of mtDNA alterations in cell and/or tissue. As mentioned, cells are polyplasmic for mtDNA, which implies that *de novo* somatic mutations in mtDNA would be in heteroplasmy, at least at beginning. Moreover, mutated mtDNA frequencies can vary dramatically between tissues (Shoffner et al. 1990; Goto et al. 1990). Actually, even in mitochondrial disorder patients, there is considerable clinical heterogeneity with mostly mtDNA mutations in heteroplasmy that are also considered highly recessive (Tuppen et al. 2010). This is particularly important for mutations causing lethal impairments that would be viable only in heteroplasmy. When heteroplasmy is present, there is a minimum critical frequency of mutated mtDNAs necessary to biochemical defects and tissue dysfunction become apparent. In humans, it has been reported that pathogenic mtDNA mutations only cause respiratory chain dysfunction when they are present above a certain threshold level, which is 60% for single large mtDNA deletions (Hayashi et al. 1991) and 90% for certain point mutations in tRNA genes (Chomyn et al. 1992). Therefore, threshold value varies for each mutation but it also differs amongst tissues according to the dependence on the oxidative metabolism presented by the tissue. It would be higher in tissues that need to obtain most energy from oxidative phosphorylation than in those that can rely on anaerobic glycolysis (Schultz and Harrington 2003). However, it is important to note that mutations in nuclear genes and in mitochondrial genes other than those in the respiratory chain also can lead to mitochondrial dysfunction. These are mainly nuclear genes encoding for respiratory chain subunits, as well as those controlling mtDNA structure and function (Leonard y Schapira 2000).

Overall, a moderate decline of respiratory chain function with age has been widely reported (Trounce et al. 1989). However, in many cases, respiratory chain deficient cells by accumulation of mtDNA alterations would represent only a part of the cells present in a tissue or organ. In addition, age-associated somatic mtDNA mutations tend to undergo clonal expansion and thereby cause focal respiratory chain deficiency. Focal respiratory chain deficiency is a ubiquitous phenomenon in human aging tissues (Müller-Höcker 1989, 1990; Trifunovic and Larsson 2008) supporting that mitochondrial dysfunction is important in human aging. Mosaic respiratory chain deficiency has been also found in many different types of aged tissues in humans including heart (Müller-Höcker 1989), skeletal muscle (Fayet et al. 2002; Bua et al. 2006; Park et al. 2009), hippocampal neurons (Cottrell et al. 2001), choroid plexus (Cottrell et al. 2001), midbrain dopaminergic neurons (Bender et al. 2006), and colon (Taylor et al. 2003). However, the responsibility of

age-associated accumulation mtDNA alterations for oxidative phosphorylation impairment has been discussed since low levels of mutated mtDNA has been found in aged humans. Still, there are evidences in favor of mtDNA effects on mitochondria function. Initially, clonal accumulation of deleted mtDNA was associated with focal respiratory chain deficiency in skeletal muscle fiber segments (Fayet et al. 2002). Similarly, it has been found that mtDNA deletions are common (Bender et al. 2006) in respiratory chain-deficient dopaminergic neurons (Reeve et al. 2009). Studies in rats, rhesus monkeys, and humans have shown that accumulation of deleted mtDNA colocalizes with respiratory chain deficiency (Wanagat et al. 2001; Bua et al. 2006). A severe respiratory chain dysfunction has been also found in cardiomyocytes from Tfam homozygous knockout mice that present an associated mtDNA depletion (Wang et al. 2001).

In humans, most published cases until now show that clonally expanded mutations are single large mtDNA deletions. The deletions differ in various respiratory chain-deficient cells of the same tissue, which is in agreement with their somatic nature (Larsson 2010). Along with deletions, clonally expanded point mutations also have been found in other tissues from aging subjects. For instance, in colonic crypts from elderly humans which show focal respiratory chain deficiency in more than 15% of all colonic crypts (Taylor et al. 2003). It has been suggested that they would be originated in the crypts stem cells and they clonally expand with division. It has been suggested that accumulated mutation type by a particular tissues depend on its mitotic activity.

Various experimental models have improved the understanding of the functional consequences of mtDNA mutations and their molecular mechanisms. Biochemical effects of mtDNA mutations have been well described in all experimental systems and are invariably characterized by lower mitochondrial respiration, compromised mtETC complex activity, and reduced ATP synthesis. However, mitochondria also play important roles in different cellular pathways beyond ATP production. These include apoptosis and nucleotide synthesis, calcium regulation (Smeitink et al. 2006; Diot et al. 2016). Therefore alterations in mitochondrial function might affect to different processes that can modulate aging at distinct levels. Oxidative stress has been studied in various animal models that accumulate mtDNA mutations with associated mitochondrial dysfunction. In Tfam homozygous knockout mice there are an initial increased ROS production as consequence of mtDNA depletion and respiratory chain dysfunction (Wang et al. 2001). In contrast, mouse strains knockout for complex I Ndufs4 protein (Kruse et al. 2008) and apoptosis inducer factor (AIF) (Pospisilik et al. 2007) do not have substantially increased ROS production or oxidative damage. Evidence of oxidative stress was almost also absent in Polγ "mutator" (Kujoth et al. 2005; Trifunovic et al. 2005; Niu et al. 2007), although there are rare exceptions (Geromel et al. 2001). In contrast, Tfam heterozygous knockout mice, which also undergo mild mtDNA depletion exhibit increased oxidative mtDNA damage susceptibility (Woo et al. 2012). In addition, apoptotic cell loss can be a common feature in respiratory chain deficiency (Trifunovic and Larsson 2008). These mutator mice displayed a massive increase in apoptosis (Kujoth et al. 2005; Trifunovic et al. 2005; Niu et al. 2007) that has been also observed in mice

conditionally knockout for Tfam that led to abolished mtDNA expression (Wang et al. 2001).

The accumulation of deficient mitochondria in a cell can lead to compensatory increase in mitochondria number. This would be mediated by the increase in mitochondrial biogenesis, which in turn leads to an increased number of mtDNA copy number in the cell. In skeletal muscle, it has been reported that ragged-red fibers, which are featured by an extraordinary accumulation of mitochondria, have very high proportions of mutated mtDNA in comparison with adjacent normal-appearing fibers (Moraes et al. 1992). It has been suggested that so massive amount of mitochondria is due to the activated mitochondrial biogenesis indeed, which is a futile response in this case. This is expected since new mtDNA molecules also harbor the same mutations, thus, many additional mitochondria remain dysfunctional. Moreover, this compensatory increase in copy number could lead to mutated mtDNA accumulation (Elson et al. 2001). Still, it has been reported that a higher number of mitochondria compensates for a decrease of oxidative phosphorylation capacity in associated to a mitochondrial myopathy in mouse skeletal muscle (Wredenberg et al. 2002; Wenz et al. 2008).

More recently, some investigations have been directed particularly to stem cells. Neural stem cells from mtDNA mutator mouse showed decreased renewal *in vitro* and quiescent pools of neural stem cells were decreased, whereas the haematopoietic stem cells showed a skewed lineage differentiation leading to anaemia and lymphopenia (Ahlqvist et al. 2012). In contrast, other model known as mtDNA "deletor" mice (Tyynismaa et al. 2005) that accumulate large-scale mtDNA deletions in postmitotic tissues and exhibited a similar late-onset respiratory chain deficiency not present any signs of premature aging as the mtDNA mutator mouse. This last probably is correlated with the fact that they have no similar somatic stem cell phenotypes (Ahlqvist et al. 2012). Therefore, consequences of mtDNA mutations in stem cells may explain at least in part the aging phenotypes.

Consequences of impaired mitochondrial respiratory chain function derived from accumulation of mtDNA mutations, combined or separately, would contribute to age-associated organ dysfunction and disease onset. Studies have suggested an association of deleted mtDNA with areas of fiber atrophy and splitting, thus, that mitochondrial dysfunction have a role in age-associated sarcopenia (Pak et al. 2003). Similarly, frequency of mtDNA mutation is higher in patients with parkinson´s disease that in age-matched controls (Bender et al. 2006). Concerning cancer, colon (Polyak et al. 1998) and prostate (Chinnery et al. 2002) cancer cases has been also associated to mtDNA mutations. Curiously, different evidences suggest that the most important factors in determining clinical symptoms are not the size and location of the deletions but tissue distribution (Zeviani et al. 1988; Moraes et al. 1995; Vielhaber et al. 2000).

The Polγ mutator mouse also results useful in this aspect since certainly shows that high levels of mtDNA mutations cause a phenotype that included shortened life-span, weight loss, osteoporosis, kyphosis, reduced subcutaneous fat, alopecia, reduced fertility, and cardiac hypertrophy (Trifunovic et al. 2004). This suggests a link between mtDNA mutations and aging phenotypes in mammals. However, the

existence of a premature aging syndrome does not necessarily implies that mtDNA mutation levels found in normal aging are high enough to cause aging-related pathology. Additional experiments to test whether a decrease in somatic mtDNA mutations extends lifespan need to be done to confirm that. In contrast, the mtDNA mutator mice show no signs of premature aging (Tyynismaa et al. 2005) in spite of similarities in mutations accumulations and mitochondrial dysfunctions. Because of between featured stem cells mentioned above (Ahlqvist et al. 2012), it has been suggested that somatic stem cell dysfunction has a crucial role of in generating the progeroid phenotype seen in mtDNA mutator mice.

In some occasions, the consequences in mitochondria and cell physiology of mtDNA mutation accumulation led enhanced aging alterations or age-associated diseases progression. Actually, abnormalities of mtDNA have been described in several diseases and high levels of deletions and point mutations cause human mitochondrial disease or syndromes**.** It has been characterised up to 250 pathogenic mtDNA mutations (point mutations and rearrangements) (Schaefer et al. 2008) causing a wide variety of diseases with a heterogeneity of phenotypes and a variable age of onset (McFarland et al. 2007). Although many mutations are heteroplasmic, there are also an increasing number of pathogenic homoplasmic mutations, often affecting just a single tissue and characterized by incomplete penetrance (McFarland et al. 2002, 2004, 2007; Temperley et al. 2003; Taylor et al. 2003; Yang et al. 2009). In concordance with previous observation, mitochondrial disorders share common cellular consequences including a decreased ATP production, an increased reliance on alternative anaerobic energy sources, and an increased production of reactive oxygen species. Regardless alteration responsible for these, studies in patients with mitochondrial disease were thus able to establish a clear cause and-effect relationship between mtDNA mutations and respiratory chain dysfunction. In addition, an increased ROS production has been described in different models with mutations associated to any of these diseases (Wong et al. 2002; Baracca et al. 2007; Li et al. 2008).

10.4 Therapies Based on Coenzyme Q Against Diseases Associated with mtDNA Alterations

Despite the role of the alterations in mtDNA in aging have not clarified yet, different treatments have been tested to retard aging or to attenuate aging consequences, which, among other possible effects, can prevent mtDNA alterations. CoQ, usually CoQ_{10}, has been used with this aim in humans and in different experimental models with this aim. Traditionally, the interest in this molecule usually comes from two main roles or activities. On the one hand, CoQ is an essential factor for cell bioenergetics as consequence of its activity as electron carrier in mitochondria. Actually, it has been proposed that an equilibrated CoQ pool may perform a better electron flow adaptation than a higher or lower CoQ pool by keeping a better mitochondrial homeostasis control (López-Lluch et al. 2010). In addition, it also seems to affect

protein complex activity and structure (López-Lluch et al. 2010). On the other hand, CoQ also is considered as an endogenously synthetized lipid-soluble antioxidant in biological membranes and it has been shown to efficiently prevent oxidation of DNA along with other macromolecules (Ernster and Forsmark-Andrée 1993). However, other roles that also result interesting for aging have been reported. These include interaction with cell signaling cascades, certain anti-inflammatory activities and even the prevention of events leading to programmed cell death. As consequence of the possible pleiotropic effect of CoQ on cell, many interventions did not aimed specifically to attenuate accumulation of mtDNA mutations. Instead, most of studies have focused on different processes related to mitochondria that have been associated with the generalized role of this organelle in aging. However, the present section of the chapter will be mainly devoted to those studies that evaluated the effect of CoQ on mtDNA.

The simplest approach has been the administration of CoQ to subjects with mitochondrial diseases or syndromes due to one or more specific mtDNA mutations (both, point mutations and deletions). The objective of this treatment was to stop the progression or to reduce some of the symptoms of these diseases. There are two features that make CoQ_{10} particularly popular in the management of patients with these disorders: its already mentioned rol as component of mtETC and its action as antioxidant, together with its well-documented safety, even at very high doses. Different trials have been carried out in this sense (Bresolin et al. 1990; Chan et al. 1998; Abe et al. 1999; Glover et al. 2010). In most of cases syndromes considered were mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), but also other rare myopathies and/or encephalopathies. In general, main parameters tested in related to mitochondrial function has been post-exercise serum (Bresolin et al. 1990; Abe et al. 1999; Glover et al. 2010) or platelets (Bresolin et al. 1990) lactate levels, which were reduced by CoQ_{10} supplementation (Bresolin et al. 1990; Abe et al. 1999; Glover et al. 2010), although not in all studies (Bresolin et al. 1990). Similarly, a study showed a reduction in lactate/pyruvate ratio that also proved to be the clinically most useful parameter in the evaluation and monitoring of mitochondrial function (Chan et al. 1998). Differences in CoQ dosage and treatment duration explain contradictory results in most cases, but in a study (Bresolin et al. 1990) observed also inter-individual differences for the same treatment that need to be clarified. It has been reported that responsiveness to treatment was apparently not related to CoQ_{10} levels in serum and platelets or to the presence or absence of mtDNA deletions (Bresolin et al. 1990). Furthermore, when they have been evaluated, it did not affect other clinically relevant variables such as strength or resting lactate (Glover et al. 2010). Therefore, these trials suggest that CoQ_{10} supplementation in relative high amount offers some improve of mitochondrial function, but the relevance for overall health is not confirmed. Likewise, most studies had a very small sample size and a rigorous placebo-controlled trial is still lacking. In addition to the above mentioned studies, there are also a set of studies using CoQ_{10} usually as a component of a "cocktail" that also includes L-carnitine, vitamin B complex, vitamin C and vitamin K_1 (Marriage et al. 2004).

To understand possible mechanisms under the effect of CoQ treatment on mitochondrial function more research is still needed. Nevertheless, an *in vitro* assay suggested that reduction of oxidative stress and inhibition of apoptosis signaling cascades could be implicated. With more detail, it has been reported that preincubation with CoQ_{10} reduced both, ROS production and activation of caspase 3, after induction by ultraviolet light in cybrids carrying mtDNA with a large-scale deletions associated to chronic progressive external ophthalmoplegia (i.e., 4366-bp and 4977-bp large-scale deletions) (Lee et al. 2005). A particular studied mitochondrial disorder is maternally inherited diabetes mellitus and deafness (MIDD) that is featured by progressive insulin secretory defect and neurosensory deafness. In a randomized-controlled trial, daily oral administration of 150 mg of CoQ_{10} for 3 years led to higher insulin secretory response than in the control group. Likewise, it improved lactate levels and prevented progressive hearing loss, although other diabetic complications and clinical symptoms remained unchanged (Suzuki et al. 1998).

In addition, dietary CoQ has shown to enhance electron transfer and ATP synthesis in some pathological situations related with aging such as cardiac failure (Rosenfeldt et al. 2005; Molyneux et al. 2009), Parkinson's disease (Beal 1999; Shults 2003; Young et al. 2007; Thomas and Beal 2010), Alzheimer's disease (Dumont et al. 2010; Yang et al. 2010; Dumont and Beal 2011) and Friedreich's ataxia (Hart et al. 2005). Although they are not specifically mitochondrial disorders, patients affected with most of these pathologies have shown a higher frequency of mtDNA alterations. In relation to these diseases, it has been reported that presence of CoQ_{10} restored the activity of impaired respiratory chain complexes I and IV in cultured fibroblasts from Parkinson´s patients. Some beneficial CoQ effects have been also observed in patients affected by HIV. This pathology is associated with alterations in the amount of mtDNA, as well as with presence of lipodystrophy and peripheral neuropathy with mitochondrial toxicity induced by reverse-transcriptase inhibitors. The administration of 100 mg of CoQ twice a day for 3 months improved the general condition and well-being in asymptomatic HIV-infected patients. However, the treatment aggravated pain in patients with peripheral neuropathy and it did not change mtDNA levels in fat and peripheral blood mononuclear cells (Rabing Christensen et al. 2004).

10.5 Coenzyme Q, Dietary Fat and Aging in Relation to mtDNA Alterations

Another approach to the study of CoQ in relation to mtDNA alterations has been the life-long dietary administration of low dosages of the molecule to rodents, in order to investigate some aspects of the interaction between nutrition and aging, mainly in relation to dietary fat. Dietary fat has been shown to be particularly interesting because of the importance of phospholipid acyl chain of mitochondrial membrane in their susceptibility to oxidative damage as well as in membrane function and

structure. This is due to the fatty acids that form them present different chemical reactivity (Pamplona 2008). Unsaturated fatty acids are more susceptible to damage from ROS molecules owing to the high presence of unstable electrons near their double bonds, and also because its sensitivity to lipid peroxidation is greater as molecules have more double bond (Bielski et al. 1983; Holman 1954). Further, they also can participate in free radical chain reactions and lipid peroxidation product would produce covalent modifications of other macromolecules as proteins and DNA. Thus, a low degree of unsaturation in the fatty acids of biological membranes would decrease their sensitivity to lipid peroxidation, which, in turn, can protect damage other lipooxidation-derivative molecules (Mataix et al. 1998). In fact, some studies in mammals have shown that fatty acids unsaturation degree in biological membranes of various tissues is negatively correlated with longevity (Pamplona 2008; Pamplona et al. 2000).

There are enough evidences indicating that fatty acids present in the diet modify the lipid profile of biological membranes, including mitochondrial membranes (Huertas et al. 1991, 1999; Ochoa et al. 2001; Quiles et al. 1999). Thus, dietary fat affects the structure and mitochondrial function, as well as its susceptibility to oxidative stress. In this sense, if we could build "customized" biological membranes depending on the type of dietary fat, maybe we could positively change the way in which the organs age. This working hypothesis represented a new approach to the study of aging from the point of view of nutrition, and had important implications for aging phenomenon study (González-Alonso et al. 2015a). This was the basis for the work of our research group in a series of experiments performed on a rat model of aging for the last 20 years. In these studies, male Wistar rats were life-long maintained on different diets with different fat sources (virgin olive oil, sunflower oil or fish oil) which notably varying in their unsaturated fatty acids profiles to evaluate how this component of the diet affected to aging of different tissues and organs. Because of mitochondria role in aging and oxidative stress, evaluations were focused on mitochondrial aspects as ultrastructural alterations, mtDNA and/or respiratory chain functionality, as well as oxidative stress (including oxidative damage and antioxidant defense components) (González-Alonso et al. 2015a). As consequence of CoQ importance in mitochondria and oxidative stress, most of the experiments were carried out by using these dietary fats without or with a supplement of CoQ_{10}.

In early interventions, rats were fed diets based on AIN-93 (Reeves 1997; Reeves et al. 1993) criteria but with different dietary fat source virgin olive oil (rich in MUFA) or sunflower oil (rich in n-6 PUFA). Animals were sacrificed at different ages to study how dietary fat and CoQ modulated aging in different tissues (Quiles et al. 2002, 2004a, b, 2005, 2006; Ochoa et al. 2003, 2011). In this context, mitochondria isolated from three different tissues, liver, heart and skeletal muscle, were compared at 6, 12, 18, and 24 months of age. Lipid peroxidation markers used (i.e. hydroperoxides levels) indicated that, in general, postmitotic tissues (i.e. heart as skeletal muscle) were more prone to suffer oxidation, but n-6 PUFA-rich diets led to a higher degree of membrane polyunsaturation and peroxidation. In addition, the degree of polyunsaturation in mitochondria was found to correlate with those in

diet, confirming a very good degree of membrane adaption to diet (Ochoa et al. 2003). Similar experiments also showed a worsening of aging effects by n-6 PUFA on different tissue and/or markers. These included total antioxidant capacity and DNA double-strand breaks which, respectively, decreased and increased in all animals as they age. In spite of all this effects of n-6 PUFA can affect to onset of some diseases, particularly those associated to aging, no changes in mean or maximal lifespan were observed (Quiles et al. 2004b).

In liver, ROS-mediated damage products (Hydroperoxides and TBARS) relative amounts were higher in 24 months old animals than in those aged 6 months but only in those receiving n-6 PUFA-rich diets, whereas rats fed virgin olive oil showed the lowest values at both ages. In most of case these levels correlates with activities of antioxidant enzymes (SOD, catalase and GPX) and concentrations of lipophilic antioxidant (α-tocopherol and CoQ). This suggests that this tissue as it ages triggers protection mechanism against oxidative stress probably as response to higher levels ROS or ROS-mediated damage products (Quiles et al. 2006). Interestingly, this study were even more focused on mitochondria an effects of diet and aging on mitochondrial ultrastructure and mtDNA were also evaluated. Namely, possible effects on mtDNA were evaluated using a particular deletion in the region encoded for mtETC complex I components (*Nd4* gene) since it has been suggested that is one of the complexes most affected by aging (Sanz et al. 2006). An age-related increase in mtDNA deletion frequency was observed in all animals but this was higher in rats fed sunflower oil. Likewise, old animals fed on n-6 PUFA rich diet displayed a lower crests number and higher circularity, factors that have been linked to a reduced functionality of mitochondria (Quiles et al. 2006). These findings, thus, revealed a relationship among ROS production and alterations of ultrastructure and mtDNA with aging at liver mitochondria in rats. But the most interesting was to see how these aspects (including accumulation of mtDNA deletion), which could be defining the appearance of aging phenotype, could be modulated through diet by choosing more or less unsaturated fat source and, which gives rise to the possibility modular aging through diet.

Based on negative consequences of n-6 PUFA intake found in above mentioned investigations, other studies were carried out where two experimental groups received similar sunflower oil-based diets, but with or without a supplementation on CoQ_{10} to reach a daily dosage of 0.7 g/kg (Ochoa et al. 2005; Quiles et al. 2004a, 2005). In heart, long-term supplementation with $CoQ_{10,}$ led to lower hydroperoxide levels, higher content of lipophilic antioxidants (α-tocopherol and coenzyme Q), and a higher catalase activity. Also, a slightly lower decrease in certain key activities for mitochondrial function when animals with age of 6, 12, or 24 months were compared (Ochoa et al. 2005). At the systemic level, an age-associated increase in nDNA strand breaks in peripheral blood lymphocytes was observed. This increase associated to age was lower in animals supplemented on CoQ_{10}. If it is assumed that main cause of such breaks is oxidative damage, this suggests that CoQ_{10} by means of both, reactive species scavenging and antioxidant recycling, protects DNA against oxidative damage. It could be possible that also can occur in mitochondria at least under certain conditions. However, this finding could also result indirectly

from of CoQ_{10} effects on mitochondria or other organelles that would reduce ROS levels and consequent attacks to nDNA (Quiles et al. 2005). In liver, similar effects on DNA double-strand breaks, CoQ levels at mitochondrial membrane have been reported. Lastly, it has also been noted that CoQ_{10}-supplemented animals reached a significantly higher mean life span and a significantly higher maximum life span (Quiles et al. 2004a). This emphasized the importance of oxidative stress, DNA damage and mitochondria in aging since CoQ has shown effect on all them (Ochoa et al. 2005; Quiles et al. 2005).

According to previous finding, it seems that life-long supplementation with CoQ_{10} of n-6 PUFA-rich diet resulted interesting to attenuate aging consequences, but it was necessary to check if CoQ_{10} led even to better results than virgin olive oil. For this reason, additional experiments similar to the previous one but including also a group fed on a virgin olive oil-based diet were carried out (Ochoa et al. 2011; Quiles et al. 2010). Thus, three diets rich in MUFA, n-6 PUFA and CoQ_{10}-supplemented n-6 PUFA were compared. Because of previous results and their importance in ROS generation and aging, mitochondria received a greater attention and mtDNA and ultrastructure were also analyzed in most of cases along with ROS and antioxidants levels. Again, the frequency of a specific deletion in mtDNA corresponding to the mETC complex I of the was used as marker of mtDNA alterations, This experimental design was used to evaluate diet and aging interaction in two tissues, both postmitotic, brain and heart.

In heart, animals fed virgin olive oil showed a lower increase in the frequency of studied mtDNA deletion than those fed sunflower oil. However, the addition of CoQ_{10} to the n-6 PUFA-rich fat source (i.e. sunflower oil) reduced the difference between young and old animals although the lowest values were present by MUFA-fed animals (Quiles et al. 2010). Concerning mitochondrial ultrastructure, dietary fat used had similar effects (Quiles et al. 2010) to those achieved in liver tissue in absence of CoQ_{10} (Quiles et al. 2006), whereas CoQ_{10} treatment led to lower mitochondrial perimeter in this case (Quiles et al. 2010). CoQ_{10} also prevented the decrease in cytochrome C oxidase activity and mtETC complex I levels suggested for old subjects fed on the same dietary fat. Therefore, it would prevent mitochondrial respiratory chain dysfunction in some degree. Aged animals receiving CoQ also showed lower hydroperoxide levels than those fed on sunflower or virgin olive oil not supplemented (Quiles et al. 2010). This would suggest that CoQ contributes to decrease oxidative stress, although there are several possible mechanisms. In any case, the effect found for dietary CoQ_{10} on either ultrastructure, mtDNA and some respiratory chain components would alleviate ROS production associated to age.

Very similar aspects were also evaluated in brain, but in this case in the experiment performed an additional group consisted in a virgin olive oil-based diet supplemented with CoQ_{10}. In this, mtDNA deletion was higher in old groups fed on n-6 PUFA-rich diets but no age-associated differences were found for animals fed virgin olive oil. However, in this case CoQ_{10} did not show effects on mtDNA deletions in animals fed on sunflower oil-based diet at 24 months. In relation to oxidative

stress markers, CoQ led to lower values of lipid peroxidation (hydroperoxides) at 24 months, although the lowest values were found in the two virgin olive oil fed groups (Ochoa et al. 2011).

These organs (heart and brain) are clear affected by aging and their alteration lead to overall health impairment reducing longevity. Moreover mitochondrial alterations and oxidative stress are key aspects in aging of these organs as it has been previously reported. Altogether, these findings revealed that CoQ, at least under certain conditions, can modulate aging effects on different tissues affecting to mtDNA, but also to mitochondrial ultrastructure and ROS production. Again, a key finding is the possibility of modulating mtDNA mutations associated to age through diet.

In more recent experiments, a third diet type has started to be compared with diets similar to previously described studies. So, as a new fat source namely fish oil, very rich in n-3 PUFA, was used. In addition, fat content was the half of the amount used in previous experiments (4% *versus* 8% w/w) according to more recent actualization of AIN93 criteria (Reeves 1997) until that moment. As previously, some additional experiments were carried out to test the effects of these diets under CoQ_{10} supplementation. Moreover, new organ/tissues, not previously studied, like pancreas and periodontum, were included in the experiments. In pancreas, it has been reported that dietary fat affected to endocrine and exocrine pancreas in a different way (Roche et al. 2014). In 24-months-old animals, n-6 PUFA rich-diets consumption was associated with a greater number of β-cells that correlated with an increase in insulin content and hyperleptinemia (Roche et al. 2014), signs that have been described in obesity, glucose intolerance, insulin resistance, disruption of adipoinsular axis or prediabetes (Sattar et al. 2008). Concerning exocrine compartment, old rats fed with n-3 PUFA-rich diets (Roche et al. 2014) led to histological features resembling those observed in pancreatic fibrosis in elderly people (Klöppel et al. 2004). In other experiments, it was observed that dietary CoQ_{10} improved endocrine pancreas structure and in particular β-cell mass from rat fed on n-6 PUFA resembling positive effects of virgin olive oil (González-Alonso et al. 2015b). Because of importance of mitochondria in this organ, CoQ_{10} effect could be mediated by effect on mtDNA previously reported (Quiles et al. 2010). However, oxidative damage or alterations of mtDNA sequence have not been directly analyzed yet. In a study focused on the pancreas of 24 months old rat fed on these diet, the profile of serum fatty acids confirmed, that animals an adaptation to the diet at 6 months of age since they resembled lipid profile of the diets. The percentages of circulating MUFA were significantly higher in rats fed virgin olive oil; the highest levels of n-6 PUFA were achieved in rats fed with sunflower oil, and the highest levels of n-3 PUFA were found in those rats fed fish oil (Roche et al. 2014; González-Alonso et al. 2015b). Moreover, the effect of this fat sources and CoQ on some bone metabolism markers at serum and age-associated alveolar bone have been also studied (Bullon et al. 2013). Alveolar bone loss is a major clinical outcome of periodontitis (Page and Kornman 1997), a disease with high prevalence in elderly people that in the last years has been associated with systemic diseases

such as atherosclerosis and metabolic syndrome that would have oxidative stress as potential link (Bullon et al. 2009, 2011). Again, feeding on an n-6 PUFA-rich diet led to worse consequences in health since it was associated to the highest age-associated alveolar bone loss (Bullon et al. 2013). Although mtDNA alterations were not directly measured, expression of genes LC3 and ATG5 that are implicated in autophagy and the biogenesis markers Tfam and PGC-1α suggests that both processes increase with aging in gingival tissue, but not in animals fed n-6 PUFA. The combination of both processes would reduce or prevent accumulation of damage in mtDNA and its possible consequences. Moreover, this effect also was associated affecting to some mtETC components and antioxidant enzymes expression. In other study, CoQ supplementation eliminated differences in age-associated alveolar bone loss among dietary groups (Varela-Lopez et al. 2015). CoQ_{10} had no effect on age-associated changes in expression of genes of autophagy markers in rats fed on n-6 PUFA-rich diet (Varela-Lopez et al. 2015). An increase in the expression of the biogenesis marker Tfam was observed in n-6 PUFA fed animals indicating that there was an increase in mitochondria and probably in mtDNA copies. Although this mechanism possibly does not reduce the accumulation of altered or mutated mtDNA molecules, it seems that it can compensate, at least in part, the associated loss of function, as it has been reported for skeletal muscle. Sumarizing all these experiments on aged rats, it could be concluded that the basis for a putative beneficial effect of CoQ on mtDNA disturbances could be the enhancement of the cellular antioxidant protection systems in cell membranes where CoQ preventing lipid peroxidation and consequently reduced oxidative stress and mtDNA damage by ROS.

Finally, in healthy humans, comparisons between CoQ_{10} supplementation to diet have been also established following a cross-over design, although only for a short period of time (4 weeks). In this regard, elderly subjects following a Mediterranean diet (rich in MUFA) supplemented and not with CoQ_{10} or a Western diet rich in SFA were studied (Yubero-Serrano et al. 2010, 2012; Gutierrez-Mariscal et al. 2011, 2014; González-Guardia et al. 2015). Some postprandial oxidative stress marker levels were reduced by CoQ_{10} addition to the MUFA-rich diet (Yubero-Serrano et al. 2010, 2012). Interestingly, dietary CoQ also improved DNA repair systems (Gutierrez-Mariscal et al. 2011; Yubero-Serrano et al. 2012). These results suggest that CoQ may also protect mtDNA against the accumulation of mutations by this mechanism in addition to the prevention of ROS-mediated damage discussed for the rat models.

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