

Effects of Two Important Components Related with Mitochondria; CoQ10 and Acetyl-L-Carnitine in Antioxidant Enzyme Activities

Ayşe Gul Mutlu and Muzaffer Dukel

Department of Biology, Art-Sciences Faculty, Mehmet Akif Ersoy University, Burdur, Turkey

Abstract: For a number of years, coenzyme Q was known for its key role in mitochondrial bioenergetics; later studies demonstrated its presence in other subcellular fractions and in plasma and extensively investigated its antioxidant role. Coenzyme Q levels are also affected during aging and neurodegenerative diseases. Very few studies have been carried out to know effects of dietary coenzyme Q in wild type animals. Acetyl-L-carnitine facilitates the uptake of acetyl-CoA into the mitochondria during fatty acid oxidation. Acetylcarnitine supplementation has beneficial effects in elderly animals and humans. In current study, the effects of coenzyme Q10 and acetyl-L-carnitine in antioxidant enzyme activities and protein amounts were investigated. The use of coenzyme Q10 and acetyl-L-carnitine, two important component related with mitochondria as food supplements have similar effects in terms of antioxidant enzyme activities and protein amount of organism.

Key words: *Drosophila*, antioxidant enzyme activities, coenzymeQ10, acetyl-L-carnitine, beneficial effect, fatty acid oxidation

INTRODUCTION

Coenzyme Q10 (CoQ10) is a widely used food supplement. Intake of CoQ10 as a food supplement increase ubiquinol levels in cell (Littarru and Tiano, 2007) and elevates the endogenous content of both CoQ9 and CoQ10 in rats and mice (Sohal *et al.*, 2006). Exogenously supplied CoQ10 can play an anti-aging function. It may do so either by acting as an antioxidant to dismutate the free radical superoxide anion or by reducing the uncoupling of reactions during electron transport that could otherwise result in superoxide anion production (Ishii *et al.*, 2004). Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. The various antioxidants exert their effect by scavenging superoxide or by activating of a battery of detoxifying/defensive proteins. The prevention of oxidation is an essential process in all the aerobic organisms as decreased antioxidant protection may lead to cytotoxicity, mutagenicity and/or carcinogenicity (Mates, 2000).

For a number of years, CoQ was known for its key role in mitochondrial bioenergetics; later studies demonstrated its presence in other subcellular fractions and in plasma and extensively investigated its antioxidant role. These two functions constitute the basis on which research supporting the clinical use of CoQ10 is founded. Furthermore, recent data reveal that CoQ10 affects expression of genes involved in human cell signalling,

metabolism and transport and some of the effects of exogenously administered CoQ10 may be due to this property. In its reduced form, CoQH₂, ubiquinol, inhibits protein and DNA oxidation but it is the effect on lipid peroxidation that has been most deeply studied (Littarru and Tiano, 2007). CoQ is a key component for, at least, three essential systems in the cell: the respiratory transport chain in mitochondria, an antioxidant component in cell membranes and a key components in the maintenance of the redox homeostasis of the cell. Due to these essential roles, deficiency of CoQ is involved in several diseases most of them associated with aging process and mainly carrying neuromuscular disorders (Quinzii *et al.*, 2007). CoQ levels are also affected during aging and neurodegenerative diseases. Very few studies have been carried out to know effects of dietary CoQ in wild type animals (Lopez-Lluch *et al.*, 2010). In different cardiovascular diseases, including cardiomyopathy, relatively low levels of Q₁₀ in myocardial tissue have been reported (Overvad *et al.*, 1999). Acetyl-L-carnitine is an ester of the trimethylated amino acid, L-carnitine and is synthesized in the brain, liver and kidney by the enzyme LAC transferase. Acetyl-L-carnitine facilitates the uptake of acetyl-CoA into the mitochondria during fatty acid oxidation (Calabrese *et al.*, 2006).

Other reported neurobiological effects of acetyl-L-carnitine include modulation of: brain energy and phospholipid metabolism; cellular macromolecules,

including neurotrophic factors and neurohormones; synaptic morphology and synaptic transmission of multiple neurotransmitters. Potential molecular mechanisms of acetyl-l-carnitine activity include: acetylation of -NH₂ and -OH functional groups in amino acids and N terminal amino acids in peptides and proteins resulting in modification of their structure, dynamics, function and turnover and acting as a molecular chaperone to larger molecules resulting in a change in the structure, molecular dynamics and function of the larger molecule (Pettegrew *et al.*, 2000).

Acetylcarnitine supplementation has beneficial effects in elderly animals and humans, including restoration of mitochondrial content and function. These effects appear to be dose-dependent (Rosca *et al.*, 2009). Acetyl-l-carnitine reverses the age-related decline in carnitine levels and improves mitochondrial beta-oxidation in a number of tissues in old rats. However, acetyl-l-carnitine supplementation does not appear to reverse the age-related decline in cardiac antioxidant status and thus may not substantially alter indices of oxidative stress (Hagen *et al.*, 2002). Acetylcarnitine is proposed as a therapeutic agent for several neurodegenerative disorders. Calabrese indicated that it may play a critical role as modulator of cellular stress response in health and disease states (Calabrese *et al.*, 2006). Clinical application of carnitine holds much promise in a range of neural disorders such as Alzheimer's disease, hepatic encephalopathy and other painful neuropathies. Topical application in dry eye offers osmoprotection and modulates immune and inflammatory responses. Carnitine has been recognized as a nutritional supplement in cardiovascular disease and there is increasing evidence that carnitine supplementation may be beneficial in treating obesity, improving glucose intolerance and total energy expenditure (Flanagan *et al.*, 2010). l-carnitine have an effective DPPH• scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total reducing power and metal chelating on ferrous ions activities (Gulcin, 2006).

The mitochondria are the principal generator of ROS during the conversion of molecular oxygen to energy production where approximately 0.4-4% of the molecular oxygen metabolized by the mitochondrial electron transport chain is converted to ROS (Lim *et al.*, 2006). Cellular damage caused by radicals may induce cancer, neurodegeneration and autoimmune disease (Rodriguez *et al.*, 2004). Toxic materials may produce ROS and generate oxidative damage on mitochondrial DNA (mtDNA) (Mutlu and Fiskin, 2009). mtDNA damages may trigger mitochondrial dysfunction. Mitochondria contribute to cardiac dysfunction and myocyte injury via

a loss of metabolic capacity and by the production and release of toxic products (Lesnefsky *et al.*, 2001). Many defense mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they inflict. Among the defenses are enzymes such as Superoxide Dismutase (SOD), catalase and Glutathione S Transferase (GST). In addition to the protective effects of endogenous enzymatic antioxidant defenses, consumption of dietary antioxidants appears to be of great importance. Fruits and vegetables, the main source of antioxidants in the diet are associated with a lowered risk of degenerative diseases (Ames *et al.*, 1993). Some expectations can be drawn from the free radical theory. Adding antioxidants to the diet which changes the balance between oxidants and antioxidants may increase longevity. Antioxidants were reported to increase longevity in some studies but not in others (Le Bourg, 2001).

Drosophila is highly amenable to scientific studies because of its short generation time, comprehensive resources for genetic manipulation and functionally conserved physiology (Shaw *et al.*, 2008). *Drosophila* have SOD, catalase, Glutathion Reductase (GR) and GST antioxidant enzyme activities but not detected Glutathion Peroxidase (GPx) (Le Bourg, 2001; Tu and Akgul, 2005).

MATERIALS AND METHODS

Experimental groups were CoQ10 application group, acetyl-l-carnitine application group and control. Wild type male *Drosophila melanogaster* were used in the experiments. Flies were fed with corn meal. Corn meal medium contained 1 L of water, 104 g corn flour, 94 g sugar, 19 g yeast, 5 g agar, 5 mL propionic acid mix. Flies were housed in glass tubes and incubated at 25°C and 12 h day-night cycle. About 31.25 mg CoQ10 (Vitamin World, Q-Sorb Coenzyme Q10) and 50 mg acetyl-l-carnitine (SIGMA A6706) were added in freshly cooked 250 mL corn meal medium for application groups. Totally 160 male flies (1 day age) were collected for enzyme activities of experimental groups. Enzyme activity assays were done in 1, 15 and 30 days age flies. Flies were homogenized individually in ultrasonic bath. Flies were placed in 2 mL tubes, PBS (pH 7.4) and protease inhibitor cocktail were used for homogenization. Flies were homogenized in ice cold water as 6×30 sec. Homogenates were centrifuged at 12000 rpm as 20 min. Supernatants were used in the experiments. About 12 flies were used for every application groups. GST and SOD activities were measured by SIGMA CS0410 GST assay kit and SIGMA 19160 SOD assay kit, respectively, according to manufacturers instructions. Catalase activity was

measured as recommended by Luck (1974) using H₂O₂ as substrate, based on determination of H₂O₂ at 240 nm (Luck, 1974). Proteins were determined by the Bradford method (Bradford Reagent SIGMA B 6916). Data were analyzed statistically by the Minitab 13. Enzyme activities and protein amount values were estimated with Kruskal Wallis test.

RESULTS AND DISCUSSION

Enzyme activities and protein amounts of CoQ, acetyl-l-carnitine and control groups were shown in Table 1. GST activities decreased with age (Fig. 1) when catalase activities didn't change significantly (Fig. 2). SOD activities were similar 1 and 15 days flies but decreased older flies (Fig. 3). About 30 days CoQ application group was statistically different from 1 and 15 days CoQ groups in terms of GST and SOD activities and protein amounts. Also protein amount values of 30 days CoQ application group and 30 days control were different (p<0.05) (Fig. 4). Catalase, GST and SOD activities of 15 days carnitine group were higher significantly than 30 days carnitine group. Also 1 day flies and 30 days carnitine applied flies were different in terms of SOD, GST activities and protein amounts. Both 30 days CoQ and 30 days carnitine groups had higher protein values than all the other groups.

Free radicals that produced during normal metabolism cause damage to macromolecules. The free radical theory of aging proposes that the organism is unable to repair all of them and that with time, unrepaired damages accumulate and put the organism at risk: in other words, free radicals provoke aging and death (Le Bourg, 2001). According to the results SOD and GST activities decreased with age. Some researchs shown that the antioxidant enzyme activities may decrease with age. The activities of antioxidant enzymes in most tissues displays an age-dependent decline (Fujimoto *et al.*, 2010; Tiana *et al.*, 1998).

The study supported the studies that reported the results like this. Even if the antioxidant enzyme activities decreased with age this reducing was clear in CoQ10 and carnitine supplemented groups. Possible explanation of the results that the CoQ and acetyl-l-carnitine may act as prooxidants. Due to its activity on ETC, CoQ has been related to the production of ROS in mitochondria by transferring directly electrons to oxygen especially at the N-site of complex III. In fact, a higher leakage of electrons from CoQ(0) site in complex III has been proposed to be one of the main causes of higher oxidative stress and aging in rat cardiomyocytes. However, the role of membrane free CoQ in production of ROS in *in vivo* conditions is under debate and the

Table 1: Catalase, GST, SOD enzyme activities and protein amounts of control and application groups

| Groups | Catalase (IU SA /mg pro±SE) | GST (IU SA /mg pro±SE) | SOD (IU SA /mg pro±SE) | Protein amounts (mg pro±SE) |
|-------------------|-----------------------------|------------------------|------------------------|-----------------------------|
| 1 day | 125.71±23.29 | 0.168±0.027* | 20.66±4.93* | 0.33±0.05* |
| 15 days control | 233.44±59.11* | 0.16±0.025* | 20.29±3.62* | 0.33±0.06* |
| 15 days CoQ | 124.28±22.21 | 0.143±0.013* | 17.85±4.60* | 0.40±0.06* |
| 15 days carnitine | 140.36±37.93* | 0.14±0.02* | 13.61±1.01* | 0.36±0.03* |
| 30 days control | 139.77±24.78 * | 0.098±0.02 | 12.72±2.96 | 0.36±0.07* |
| 30 days CoQ | 95.09±24.64 | 0.077±0.01 | 8.65±1.12 | 0.53±0.03 |
| 30 days carnitine | 67.19±8.58 | 0.07±0.01 | 8.69±0.64 | 0.55±0.04 |

*Values statistically different from CoQ-30 days (p<0.05), IU SA/mg pro: Unit Specific Activity/miligram protein, SE: Standard Error

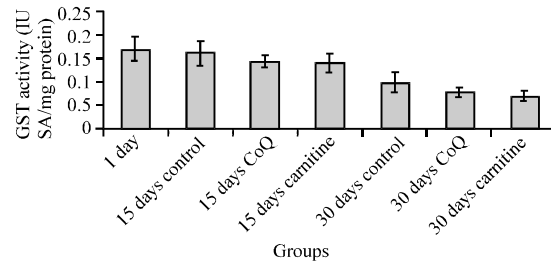


Fig. 1: GST enzyme activities of control and application groups

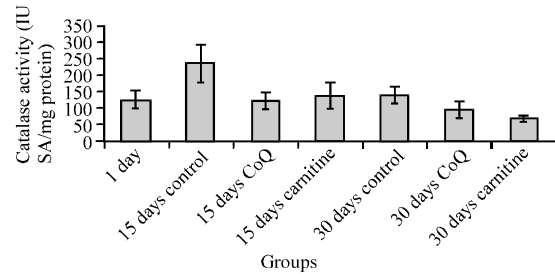


Fig. 2: Catalase enzyme activities of control and application groups

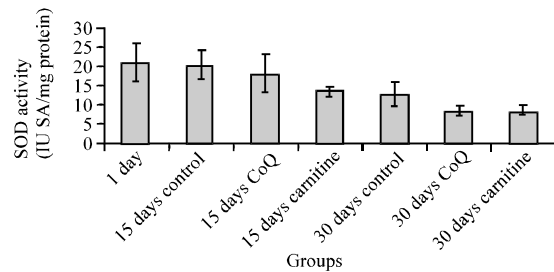


Fig. 3: SOD enzyme activities of control and application groups

importance of CoQ levels in mitochondria in ROS production is controversial (Lopez-Lluch *et al.*, 2010). Lopez Lluch reported that the increase of CoQ10 levels in mitochondrial membranes does not increase ROS

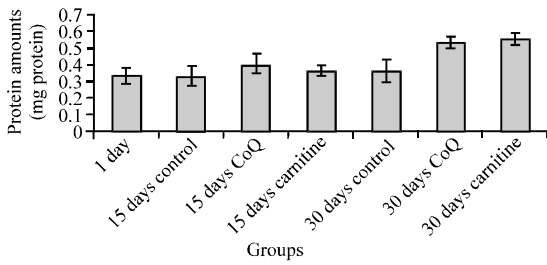


Fig. 4: Protein amounts of control and application groups

production in human cells whereas shorter forms of CoQ such as CoQ6, do increase ROS. These results indicate that high CoQ levels are not the only reason for ROS production but the nature of the exogenous CoQ form which can affect ROS production by interfering with normal electron transport chain activity. However, CoQ-deficiency in human fibroblasts can produce either high or low ROS levels indicating that the reason for electron leak process is more complex than CoQ concentration (Lopez-Lluch *et al.*, 2010). Coenzyme Q₁₀ has a central role acting as a prooxidant in the generation of H₂O₂ (Linnane and Eastwood, 2006; Linnane *et al.*, 2007). It is the prooxidant activity of the so-called antioxidants that may be responsible for previously claimed benefits for high doses of oxido-reduction nutritional supplements such as alpha lipoic acid and coenzyme Q₁₀. Oxygen-free radical formation is essential for the biological function and is not a direct causation of the mammalian aging process; aging is a multisystem stochastic process (Linnane and Eastwood, 2006). Furthermore, lifelong dietary CoQ10 supplementation decrease Q9/Q10 ratio in rat liver membranes, attenuates aging-related decrease of glutathione-dependent antioxidant activities such as cytosolic GST (Lopez-Lluch *et al.*, 2010).

CoQ10 and acetyl-l-carnitine had similar effects in antioxidant enzyme activities and protein amounts according to the results. Carnitine and its esters prevent toxic accumulations of fatty acids in the cellular cytoplasm and of acyl CoA in the mitochondria while providing acetyl CoA for mitochondrial energy production (Werbach, 2000). Rosca suggested that acetyl-l-carnitine leads to specific increase in mitochondrial gene expression and mitochondrial protein synthesis via acetylation of mitochondrial proteins (Rosca *et al.*, 2009). However, acetyl-l-carnitine improves mitochondrial beta-oxidation (Hagen *et al.*, 2002). This process may produce free radicals. Acetyl-l-carnitine increases cellular oxygen consumption which declines with age to the level of young rats (Hagen *et al.*, 1998). Hagen also reported that the oxidant production per oxygen consumed is ~30% higher in acetyl-l-carnitine treated rats than in untreated

old rats (Hagen *et al.*, 1998). Both 30 days CoQ and 30 days carnitine groups had significantly higher protein amounts than the others. These results indicated that acetyl-l-carnitine and CoQ as food supplements increase protein amounts of organisms. Iossa reported that acetyl-l-carnitine increased protein amounts in old rats (Iossa *et al.*, 2002). In the study, CoQ shown the similar effect. According to Lopez-Lluch, serum albumin which decreases with age in the rat is significantly increase by CoQ10 supplementation. Lifelong dietary supplementation with CoQ10 also induce significant modifications of several proteins in plasma (Lopez-Lluch *et al.*, 2010).

The use of CoQ10 and acetyl-l-carnitine, two important component related with mitochondria as food supplements, have similar effects in terms of antioxidant enzyme activities and protein amount of organism. According to these results, CoQ and acetyl-l-carnitine have positive effects on protein contents of organisms. However, decreasing of antioxidant enzyme activities in CoQ10 and acetyl-l-carnitine application groups, especially in older flies (30 days), suggests prooxidant activity for these materials. Low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signaling and defense against micro-organisms. Nevertheless, higher amounts of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia and failures in immunity and endocrine functions (Mates *et al.*, 1999).

CONCLUSION

According to these results, coenzyme Q10 and acetyl-l-carnitine have positive effects on protein contents of organisms. However, decreasing of antioxidant enzyme activities in CoQ10 and acetyl-l-carnitine application groups, especially in older flies, suggests prooxidant activity for these materials.

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