The impact of coenzyme Q10 supplementation on antioxidant status of mouse model of andropause

Zahra SOURI1,*, Ali BIDMESHKI POUR1 and Isaac KARIMI2

1. Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
2. Laboratory of Molecular and Cellular Biology 1214, School of Veterinary Medicine, Razi University, Kermanshah, Iran
*Corresponding author, Z. Souri, Tel.: +98 83 37243688; Fax: +98 83 38323728, Email: zahrasouri4@gmail.com

Abstract. Aging men experience a decline in the concentration of androgens specially testosterone. This decline could cause oxidative stress which potentially leads to immunosenescence. Coenzyme Q10 (CoQ10) could have beneficial effects on immunosenescence incidence in aging men. To investigate whether testosterone deficiency alters total antioxidant capacity (TAC) of plasma after orchidectomy and could exogenous CoQ10 affect these alterations, we supplemented andropause mouse with three different levels of dietary CoQ10 and evaluated the TAC of plasma. 36 adult male mice were assigned to six groups, namely normal control (NC) group receiving normal chow diet, normal mice receiving 125 mg/kg/day of CoQ10 (C125), bilateral orchidectomized (BOX) group receiving normal chow diet, and three other orchidectomized groups, namely BOX 125, 250 & 500 groups receiving 125, 250, and 500 mg/kg/day of CoQ10 mixed with normal chow diet. After 96 days, blood samples were collected and the plasma was kept on -20°C until use. Testosterone and estrogen were measured using ELISA kits while the TAC of plasma was measured using the DPPH method. Testosterone levels decreased non-significantly in BOX group compared to that of NC group while estrogen increased non-significantly in BOX groups compared to the NC group (p>0.05); also, TAC of plasma decreased non-significantly in BOX group compared to that of NC group (p>0.05), while this value in orchidectomized groups receiving different doses of CoQ10 had increased significantly compared to NC group (p<0.05). Dietary supplementation of CoQ10 increased TAC of plasma, thus CoQ10 could be beneficial to make a stronger shield against reactive oxygen species and probably lower the immunosenescence incidence in aging males.

Key words: Coenzyme Q10, antioxidant, andropause, immunosenescence, mice.

Introduction

Aging is a very complex process that comprises a lot of biological functions of the organism, resulting in an increased susceptibility to disease and death (Fulop et al. 2012). A number of studies have shown that in most men there is a slow decline in steroid synthesis, especially testosterone levels with aging, even in the absence of disease (Balasubramanian et al. 2012). Moreover, aging is actually associated with a decline in the normal functioning of the immune system. This contributes to poorer vaccine responses and the increased incidence of infection and malignancy seen in the elderly (Simpson et al. 2012). The decline in serum testosterone levels with aging is due to the impaired testicular production of testosterone and hypothalamic secretion of gonadotropin-releasing hormone (GnRH) resulting in inadequate stimulation of luteinizing hormone (LH) secretion by the pituitary gland (Matsumoto 2002, Zirkin & Tenover 2012).

As senescence occurs several distinctive changes arise in the cell such as increasing the expression of some senescent biomarkers, including increased expression of P53, P21 and P16 and other cyclin-dependent inhibitors such as P27 and P15 (Rufini et al. 2013). Changes in the immune system are part of the normal aging process. Immunosenescence, the term commonly used to describe the age-acquired dysfunctional immunity, contributes to a less-than-ideal immune response to many antigenic stimuli (i.e., from pathogens, vaccines, and diseases) compared to their younger counterparts. In fact, sex hormones modulate a large variety of phenomena involved in the immune response (Casto et al. 2001, Aw et al. 2007, Nunn et al. 2009).

The CoQ10 is the only endogenous synthetic natural fat-soluble redox lipid. It is localized into hydrophobic portions of cellular membranes as well as an intracellular distribution (Rötig et al. 2000). The chemical nomenclature of CoQ10 is 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone that is naturally in the trans-configuration (Bhagavan and Cho 2006) (Fig. 1). Approximately half of the body’s CoQ10 is obtained through dietary fat ingestion, whereas the remainder results from endogenous synthesis (Marcoff & Thompson 2007). The CoQ10 has several biological functions, for example, it serves as a cofactor of uncoupling proteins and also has an impact on gene expression (Schmelzer & Döring 2012) (Fig. 1). CoQ10 also has effects on genetic stability and can change the result of DNA damage gene mutation, telomere shortening and epigenetic modifications (Schmelzer & Döring 2012).

It is known that low testosterone in human can cause an
oxidative stress condition. This condition could be associated with alterations in the antioxidant defense mechanisms (von Schantz et al. 1999, Alonso-Alvarez et al. 2007, Turner & Lysaik 2008). The aim of the present study was to investigate the effect of dietary supplementation of CoQ10, as a strong antioxidant on total antioxidant status in andropause mice.

Material and methods

Animal subjects
The ethical committee of Razi University, Kermanshah, Iran was reviewed and approved this research. Adult weight- and age-matched healthy male NMRI mice (n=56), with an average 6 week old and 30g body weights. The animals were housed in standard plastic cages (6 mice/cage) for 96 days and acclimatized for 3 weeks. Food (Dan-e- pars Co., Kermanshah, Iran) and water were available ad libitum throughout the study, and the colony room was maintained at 22 ±1°C and humidity 55%, on a 12 hour light-dark cycle beginning at 0600 h.

Mouse model of andropause
At 6 to 7 weeks of age, male NMRI mice were bilaterally orchidectomized (BOX) or received a sham operation under deep anesthesia with intraperitoneally (i.p.) injection of ketamine (80 mg/kg; Alfasan Co., Netherland) / diazepam (0.5 mg/kg; Chemi. Darou Co., Iran) cocktail. The mice were allowed two weeks to recover from the operations before they were fed with CoQ10 supplemented diet.

During the experiment, mice were randomly assigned to six groups: Normal control group (NC) that fed a standard diet; positive control group (C125) that received 125 mg/kg/day CoQ10 in its diet; BOX group that fed a standard diet; and three other BOX groups, namely BOX125, BOX250, and BOX500 which received 125, 250, and 500 mg/kg/day CoQ10 in their diets, respectively.

Testosterone and Estrogen measurement
Blood was collected by heart puncture, centrifuged and plasma collected and frozen at −80°C within 30 minutes. Testosterone and estrogen were measured in plasma using ELISA kit (Diametra, Italy) according to manufacturer protocol.

Estimation of total antioxidant status (TAS) by DPPH assay
One analytical method for evaluating the sum of TAC of the plasma is the DPPH (2, 2-diphenyl-1-picryl hydrazyl) method as described by (Mahlouz et al. 2009). Briefly, 0.1 ml of plasma was deproteinated by adding 1 ml of methanol 97% (Kimia toos Co. Iran), vortexed for 30 s and centrifuged at 3000 rpm for 30 min. 1.5 ml of methanol and 0.5 ml of stable free radical (DPPH) 0.2 mM that prepared in methanol were added to the supernatant, mixed thoroughly and absorbance was read at 517 nm against blank. Ascorbic acid was employed as a reference standard. The standard graph was plotted using different concentrations of ascorbic acid and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Hematology profile (White blood cell (WBC) staining and differential WBC counting)
One drop of tail blood was collected from each sample on day 96, then a drop of blood was placed, and using a spreader the drop spread out quickly; then the film was dried rapidly. Before staining, the blood films were needed to be fixed with acetone free methyl alcohol for 0.5 to 1 minute. The blood film was fixed with methyl alcohol for 2 minutes. Giemsa stain diluted 1:9 with distilled water and poured over the smear for 15 minutes. Then, films washed off with water. Finally, The dry and stained film was examined without a coverslip under oil immersion objective. A total of 100 cells was counted in which every white cell seen, was recorded.

Results

Body weight growth
Growth generally includes an increase in height, length, and weight that occurs when an animal is given adequate food, water and shelter (Swatland 1994). The effect of bilaterally orchidectomy and CoQ10 supplementation on body weight of different groups is shown in Table 1. All the animals were healthy throughout the experimental period and CoQ10-treated groups were apparently more motivated and hopeful (data not shown). It was observed that bilaterally orchidectomy and dietary CoQ10 did not influence on body weight significantly (p>0.05), although C125 group was heavier than NC group and BOX 500, BOX 250 and BOX125 were heavier than the BOX group showing that CoQ10 supplementation can cause an increase tendency of body weight. Finally the BOX group was heavier than the NC group, indicating that similar to dietary CoQ10, bilaterally orchidectomy can cause weight gain during ten week (Fig. 2).

The results of hormone assay
Table 2 represents the results of testosterone and estrogen levels in CoQ10-treated BOX mice. Because of wide individual diversity due to social hierarchy in each group no significant differences were found among the groups. Testosterone levels in all the bilaterally orchidectomized groups,

Table 1. The average body weight of experimental groups during 10 weeks of intervention.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Average body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>31.90±0.38</td>
</tr>
<tr>
<td>C125</td>
<td>33.16±0.78</td>
</tr>
<tr>
<td>BOX</td>
<td>33.50±0.43</td>
</tr>
<tr>
<td>BOX125</td>
<td>32.38±0.71</td>
</tr>
<tr>
<td>BOX250</td>
<td>34.51±0.70</td>
</tr>
<tr>
<td>BOX500</td>
<td>34.18±0.97</td>
</tr>
</tbody>
</table>

NC: normal control, C125: normal controls receiving 125mg/kg/day of CoQ10, BOX, BOX 125, BOX250 and BOX500: bilaterally orchidectomized groups, receiving 0.0, 125, 250, and 500 mg/kg/day of CoQ10, respectively. Data show mean (± SE) of six mice/group.

Figure 2. The gradual live body weight changes of experimental groups during 10 weeks of intervention. NC: normal control, C125: normal controls receiving 125mg/kg/day of CoQ10, BOX, BOX 125, BOX250 and BOX500: bilaterally orchidectomized groups, receiving 0.0, 125, 250, and 500 mg/kg/day of CoQ10, respectively. Data show mean (± SE) of six mice/group.
Table 2. The steroid hormone profile of experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>C125</th>
<th>BOX</th>
<th>BOX125</th>
<th>BOX250</th>
<th>BOX500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>2.42±2.21</td>
<td>2.05±1.90</td>
<td>0.29±0.06</td>
<td>0.21±0.08</td>
<td>0.21±0.08</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
<td>1.60±1.42</td>
<td>0.38±0.11</td>
<td>4.21±3.47</td>
<td>0.22±0.02</td>
<td>0.97±0.49</td>
<td>0.3±0.04</td>
</tr>
<tr>
<td>*T/E ratio</td>
<td>1.17±0.71</td>
<td>9.7±6.43</td>
<td>1.01±0.38</td>
<td>0.97±0.34</td>
<td>0.35±0.15</td>
<td>0.67±0.03</td>
</tr>
</tbody>
</table>

NC: normal control, C125: normal controls receiving 125mg/kg/day of CoQ10, BOX, BOX125, BOX250 and BOX500: bilaterally orchidectomized groups, receiving 0.0, 125, 250, and 500 mg/kg/day of CoQ10, respectively. *T/E ratio: testosterone to estrogen ratio. Data show mean (± SE) of six mice/group.

Table 3. The differential white blood cell count of experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>C125</th>
<th>BOX</th>
<th>BOX125</th>
<th>BOX250</th>
<th>BOX500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>27.80±3.40ab</td>
<td>39.25±4.55a</td>
<td>23.50±5.01ab</td>
<td>26.17±3.06ab</td>
<td>22.20±1.98b</td>
<td>28.33±2.12ab</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>69.80±3.47ab</td>
<td>59.50±4.70b</td>
<td>75.50±5.27ab</td>
<td>73.33±3.11ab</td>
<td>77.60±1.86a</td>
<td>71.50±2.14ab</td>
</tr>
<tr>
<td>Neutrophil/Lymphocyte</td>
<td>0.41±0.07ab</td>
<td>0.69±0.13a</td>
<td>0.34±0.1 ab</td>
<td>0.36±0.05 ab</td>
<td>0.28±0.03b</td>
<td>0.40±0.04ab</td>
</tr>
<tr>
<td>Monocyte</td>
<td>2±0.00</td>
<td>1.66±0.66</td>
<td>1.50±0.28</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NC: normal control, C125: normal controls receiving 125mg/kg/day of CoQ10, BOX, BOX125, BOX250 and BOX500: bilaterally orchidectomized groups, receiving 0.0, 125, 250, and 500 mg/kg/day of CoQ10, respectively. Data show mean (± SE) of six mice/group. Values in each group with different letter are significantly different (P<0.05).

The differential leukocyte count
Table 3 represents the differential leukocyte count in CoQ10-treated bilaterally orchidectomized mice. The only significant differences in neutrophil percentage were seen in the BOX250 group with the lowest neutrophil percent, and the C125 group with the highest neutrophil percent (p<0.05). The BOX, BOX125 and BOX250 groups had non-significantly lower percentage of neutrophil than that of the NC group, while, BOX125, BOX250, BOX 500 all had lower testosterone to estrogen ratio compared to the BOX group (p<0.05) (Table 2).

The effect of CoQ10 on hormonal balance and body weight in mouse model of andropause
Aging is a complex progressive physiological alteration in all organisms which is defined as a process that is manifested at genetic, molecular, cellular, organ and system levels (Pandey & Rizvi 2010). This normal physiological condition in term
could cause decelerated effects in different tissues namely gonadal tissues. Aging in men has gradually effects on gonads referred to by some as “androgen deficiency in the aging male (ADAM),” “partial androgen deficiency in the aging male (PADAM),” or “aging-associated androgen deficiency (AAAD)” (Matsumoto 2002). This phenomenon is associated with a gradual and progressive decline in serum testosterone levels at a rate of approximately 1% per year (Matsumoto 2002). As a result, 20% of men older than 60 and 50% of men older than 80 years of age have serum total testosterone levels below the normal range (Matsumoto 2002).

The decline in serum testosterone levels with aging has multiple reasons, but it is mainly associated with the impaired testis production of testosterone, which is caused by a decrease in Leydig cell number (Pantalone & Faiman 2012). Other studies showed that there are trinucleotide CAG repeats in the androgen receptor gene with variable length, which the length is associated with differences in transcriptional activity. Older men with lower serum testosterone levels have an androgen receptor genotype characterized by a shorter CAG repeat length, suggesting overall greater androgen activity. This may be an intrinsic mechanism that underlies the physiological decline in serum testosterone levels with aging (Mäkinen et al. 2005). In the current study, the level of plasma testosterone in BOX groups was lower than that of seeing in NC, indicating that the main source of testosterone production is the testis but as it was no significant. We can conclude that testosterone can have some other extra-gonadal sources such as the adrenals and adipose tissue and in addition a wide diversity in our groups is governed. By the side CoQ10 supplementation has not any significant effects on extra-gonadal sources of testosterone.

Testosterone decline during aging causes some alterations in body such as diminished energy and muscle strength, reduced sexual function, virility, and fertility, depressed mood and decreased cognitive function, skin thickness, bone mineral density resulting in osteoporosis, and decrease in body hair along with related metabolic changes (Matsumoto 2002). Aging is associated with increased fat mass and also associated with increased conversion of androgens to estrogens (Mäkinen et al. 2005). Testosterone is actively metabolized to the potent estrogen, estradiol (E2), by the activity of aromatase, which is located primarily in adipose tissue therefore testosterone deficiency may be due to increase in the aromatization of testosterone to E2 (Mäkinen et al. 2005). In agreement with aforementioned findings, our results cautiously showing that the BOX group, in contrast to removing the testes was heavier than NC group and its plasma estrogen level (4.21±3.47) pg/ml group is higher than the NC (1.60±1.42) pg/ml group may be due to increased adipose tissue mass.

The Effect of CoQ10 on TAC of plasma
To prevent the toxicity of reactive oxygen species (ROS), cells are equipped with multiple antioxidant defense mechanisms (Tam et al. 2003). The first line of defense includes enzymes such as superoxide dismutase’s, glutathione peroxidase 1 (Gpx1), catalase, peroxiredoxin 5 (Pdx5) and glutathione S-transferases (GSTs) (Sies 2007). The second line of cellular defense against antioxidants involves generation of intracellular reducing power, such as the reduced form of glutathione and NAD(P)H, within the cell. Enzymes such as glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), gamma-glutamyl transpeptidase (GGTP), and glutathione synthetase (GS) play major interactive roles in the replenishment of cellular reducing power (Sies 2007).
Other antioxidant molecules include thioredoxin (Trx), a protein thiol that directly detoxifies ROS and serves as an electron donor to other antioxidant enzymes, including TrxR1 (Tam et al. 2003). Early studies believed that urate (35–65%), plasma proteins (10–50%), ascorbate (0–24%), and tocopherol (5–10%) could be the majority of antioxidant defenses in plasma *in vitro* but they did not have any explanation for *in vivo*, so for this the term total antioxidant capacity, or TAC, emerged to give the concept of the “cumulative action of all the antioxidants present in plasma and body fluids” (Sies 2007).

In the present study, TAC in BOX group compared to NC group was decreased non-significantly because CoQ10 levels decrease in castrated mice and hypogonadism represents a condition of oxidative stress (Mancini et al. 2011); also, TAC in BOX groups using CoQ10 had improved indicating that it has altered the plasma redox balance toward a more reductive and less pro-oxidant environment (Gutierrez-Mariscal et al. 2012). TAC of C125 was lower than NC group because CoQ10 has altered the plasma redox balance toward a more reductive and less pro-oxidant environment (Gutierrez-Mariscal et al. 2012). TAC of C125 was lower than NC because it is toxic in testosterone existence because of enough endogenous CoQ10 in plasma (Mancini et al. 2011) and could be a major source of O2 and H2O2 generation (Kwong et al. 2002). TAC of BOX500 was lower than other BOX supplemented groups maybe because high dosages of CoQ10 are not efficient. TAC of plasma has increased significantly in the supplemented groups as CoQ10 dose has increased giving CoQ10 the power to improve antioxidant mechanisms in plasma. TAC was similar in NC and C125 groups. This result obtained in C125 group may be due to its normal antioxidant status so mice in this group really would not have needed extra exogenous antioxidant supplementation and this excess intake has been a toxic squeal for them. So we suggest this idea for the first time that before any CoQ10 prescription, patients must measure their plasma antioxidant capacity and in term of need, use exogenous CoQ10.

In conclusion, dietary supplementation of CoQ10 increased TAC of plasma, thus CoQ10 could be beneficial to make a compress and stronger shield against reactive oxygen species and other DNA damaging agents which would appear after exposure to minor injuries such as sterile implant imbalance that occurred post-gonadectomy in immune cells of mice and probably lower the immunosenesence incidence in aging males.

References
