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Mónica Santos-González, Consuelo Gómez Díaz, Plácido Navas, José Manue Villalba. Modifications of plasma proteome in long-lived rats fed on a coenzyme Q10 -supplemented diet. *Experimental Gerontology*, 2007, 42 (8), pp.798. 10.1016/j.exger.2007.04.013 . hal-00499015

**HAL Id: hal-00499015**

**<https://hal.science/hal-00499015>**

Submitted on 9 Jul 2010

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## Accepted Manuscript

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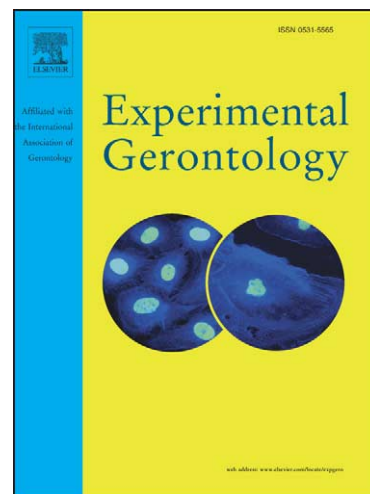
PII: S0531-5565(07)00092-7  
DOI: [10.1016/j.exger.2007.04.013](https://doi.org/10.1016/j.exger.2007.04.013)  
Reference: EXG 8339

To appear in: *Experimental Gerontology*

Received Date: 3 March 2007  
Accepted Date: 17 April 2007

Please cite this article as: Santos-González, M., Díaz, C.G., Navas, P., Villalba, J.M., Modifications of plasma proteome in long-lived rats fed on a coenzyme Q10 -supplemented diet, *Experimental Gerontology* (2007), doi: [10.1016/j.exger.2007.04.013](https://doi.org/10.1016/j.exger.2007.04.013)

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For: Experimental Gerontology

**MODIFICATIONS OF PLASMA PROTEOME IN LONG-LIVED RATS FED  
ON A COENZYME Q<sub>10</sub>-SUPPLEMENTED DIET**

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**Running title:** Plasma proteome, aging and dietary coenzyme Q

**Key words:** Aging / Coenzyme Q / Diet / Lifespan / Plasma proteome / Rat

**ABSTRACT**

Dietary coenzyme Q<sub>10</sub> prolongs lifespan of rats fed on a PUFA $n$ -6-enriched diet. Our aim was to analyze changes in the levels of plasma proteins of rats fed on a PUFA $n$ -6 plus coenzyme Q<sub>10</sub>-based diet. This approach could give novel insights into the mechanisms of lifespan extension by dietary coenzyme Q<sub>10</sub> in the rat. Serum albumin, which decreases with aging in the rat, was significantly increased by coenzyme Q<sub>10</sub> supplementation both at 6 and 24 months. After depletion of the most abundant proteins by affinity chromatography, levels of less abundant plasma proteins were also studied by using 2D-electrophoresis and MALDI-TOF mass fingerprinting analysis. Our results have shown that lifelong dietary supplementation with coenzyme Q<sub>10</sub> induced significant decreases of plasma hemopexin, apolipoprotein H and inter-alpha-inhibitor H4P heavy chain (at both 6 and 24 months), preprohaptoglobin, fibrinogen  $\gamma$ -chain precursor, and fetuin-like protein (at 6 months), and alpha-1-antitrypsin precursor and type II peroxiredoxin (at 24 months). On the other hand, coenzyme Q<sub>10</sub>-supplementation resulted in significant increases of serine protease inhibitor 3, vitamin D-binding protein (at 6 months), and Apo A-I (at 24 months). Our results support a beneficial role of dietary coenzyme Q<sub>10</sub> decreasing oxidative stress and cardiovascular risk, and modulating inflammation during aging.

## 1. INTRODUCTION

Aging is an endogenous process characterized by a progressive loss of function with decreased fertility, increased mortality and augmented susceptibility to the onset of age-related diseases (Kirkwood and Austad, 2000). Among the different theories that have been elaborated over the past 35 years to explain the causes of aging, the oxidative damage theory of aging is one of the most accepted today. This theory postulates that aging is the result of the accumulation of cellular and tissue damage caused by the continuous and endogenous generation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals, mainly of a mitochondrial and plasma membrane origins (Wallace, 1992; Barja and Herrero, 2000; Sohal, 2002; Sohal et al., 2002; Harman, 2003; Hekimi and Guarente, 2003; Barja, 2004; Schriener et al., 2005). This theory has gained a significant support with the recent demonstration that overexpression of mitochondria-targeted catalase prevents ROS production and increases life span in transgenic mice (Schriener et al., 2005), and an increase of mitochondrial biogenesis with higher bioenergetic efficiency in supporting calorie restriction effect on lifespan extension (Baur et al., 2006).

Currently there is a great interest in the development of anti-aging therapies. Since oxidative stress is involved in aging, it is considered that any strategy to reduce oxidative damage would have significant anti-aging effects (Quiles et al., 2004). From a practical and therapeutic point of view, dietary modifications may be a feasible approach to counteract the deleterious effects of prooxidants during aging. The impact of diet and dietary constituents on aging and age-associated diseases is well documented, mostly regarding calorie restriction and antioxidant nutrient supplementation (Finkel and Holbrook, 2000; Sohal et al., 2002; Finkel, 2003; Howitz et al., 2003).

Coenzyme Q (CoQ, ubiquinone) (2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone) is a prenylated benzoquinone that is present in all cellular membranes

and in high and low density lipoproteins as well. CoQ participates as an electron carrier in both mitochondrial and extramitochondrial membranes and, in its hydroquinone form (ubiquinol), it is a powerful antioxidant (Turunen et al., 2004). It has been recently demonstrated that lifelong supplementation with CoQ<sub>10</sub> produced significant increases of mean and maximum life span in rats fed a polyunsaturated fatty acids (PUFA)-enriched diet (Quiles et al., 2004). Compared to rats fed lifelong on a PUFA-alone diet, animals that were fed on a PUFA<sub>n-6</sub> plus CoQ<sub>10</sub> diet showed significantly higher levels of CoQ<sub>10</sub> in liver mitochondria and plasma membrane, less DNA double-strand breaks in blood lymphocytes, lower oxidative stress and improved mitochondrial function in the heart, and enhanced glutathione-dependent antioxidant capacity in liver (Quiles et al., 2004; Ochoa et al., 2005; Bello et al., 2005).

These previous studies were focused to the biochemical analyses of classical antioxidant proteins that have established roles in oxidative stress resistance. However, it is clear that changes in many other proteins, still to be identified, could be also related to the observed increase of longevity in CoQ<sub>10</sub>-supplemented rats. The proteomic approach offers an unbiased way to study changes in protein levels induced by different experimental conditions. This approach has revealed successful for the identification of novel mechanisms of cell responses against oxidative stress (Keightley et al., 2004). A major challenge of proteome research is detecting clinically useful biomarkers of disease, treatment response and aging (Anderson, 2005). Serum and plasma offer particularly promising resources for biomarker discovery because collection of these samples is minimally invasive and the blood is thought to contain the majority of protein constituents found in the body (Echan et al., 2005).

To better understand the possible mechanisms of lifespan extension by CoQ<sub>10</sub> in rats fed a PUFA-enriched diet, we have initiated a proteomic study to identify plasma proteins whose levels are altered due to CoQ<sub>10</sub>-supplementation during aging. Our results support a potential beneficial role of CoQ<sub>10</sub> decreasing oxidative stress and cardiovascular risk, and modulating inflammation during aging

## 2. MATERIALS AND METHODS

### 2.1 *Animals, diets and blood samples*

Animals and diets used in this work were the same as those utilized in previous works carried out by the group of Prof. José Mataix at the Universidad de Granada, Spain (Quiles et al., 2004; Ochoa et al., 2005) and by ours at the University of Córdoba, Spain (Gómez-Díaz et al., 2003; Bello et al., 2005). Briefly, groups of 10 male Wistar rats (*Rattus norvegicus*) initially weighing 80-90 g were housed under standard conditions and maintained on a 12 h light/12 h darkness cycle and at a temperature of  $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Animals were provided *ad libitum* access to water and a fresh semi-synthetic and isoenergetic diet with 8% fat (containing 61% of total fatty acids as PUFA) over a 24 months period. Animals were randomly assigned into two groups which differed in the dietary supplementation or not with CoQ<sub>10</sub> (intake of 0.7 mg/kg/day). Eight rats per group were sacrificed by cervical dislocation and further decapitation at 6 months (young) or 24 months (old) from the start of the experiment. Blood was collected by decantation onto glass tubes coated with lithium heparin and plasma was then recovered by centrifugation. No evidence of haemolysis was observed. Samples were immediately frozen in liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  for later analysis. Animals were handled at the facilities of the University of Granada (Spain) according with guidelines of Spanish Society for Laboratory animals and the experiment had the approval of the Ethical Committee of the University of Granada.

### 2.2 *Coenzyme Q determinations*

Plasma samples (about 50  $\mu\text{l}$ ) were mixed with an equal volume of 2% SDS. Afterwards, four volumes of 95% ethanol-5% isopropanol were added and the mixture was vigorously vortexed. CoQ was recovered from SDS-alcoholic solution by extraction with ten volumes of hexane. After hexane evaporation under vacuum, the lipid extract was dissolved in ethanol and used for quantification of CoQ<sub>9</sub> and CoQ<sub>10</sub> by

reversed-phase HPLC. Separation was accomplished at 1 ml/min with a C18 column (5- $\mu$ m particle, 5 x 0.45 cm) and a mobile phase composed of a mixture of methanol and n-propanol (1:1) containing lithium perchlorate (2.12 g/L). Monitoring was carried out with a Coulochem II electrochemical detector (ESA, Chelmsford, MA) fitted with a Model 5010 analytical cell with the electrodes set at potentials of -500 mV and +300 mV. Quinones were detected from the signal obtained at the second electrode. Concentrations were calculated by integration of peak areas and comparison with external standards (Bello et al., 2005). Since CoQ<sub>9</sub> is the predominant CoQ homolog in the rat, CoQ<sub>10</sub> supplementation was assessed by a decrease in the CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio.

### ***2.3 One-dimensional SDS-electrophoresis***

For protein analysis, plasma samples were pooled according to supplementation group and duration of dietary intervention (n=8). Protein content was measured by the dye binding method with bovine  $\gamma$ -globulin as standard. Levels of albumin in plasma were assessed by quantifying the intensity of the 66 kDa band in gels obtained after separation of 3  $\mu$ g plasma protein by SDS-discontinuous electrophoresis (10% polyacrylamide). Electrophoresis was developed at a constant voltage of 200 V using a MINI-PROTEAN 3 Cell (BioRad) and gels were stained with colloidal Coomassie stain (BioSafe, Bio-Rad). Gel images were obtained using a GS-800 Calibrated Densitometer (Bio-Rad) and quantification of the albumin band was carried out with Quantity One software (Bio-Rad). Results were expressed as optical density (OD) x mm<sup>2</sup>.

### ***2.4 Proteomic analysis***

Aliquots of pooled samples (60  $\mu$ l) were depleted from albumin and immunoglobulins using commercial disposable affinity columns (Proteoextract Albumin/IgG removal kit, Calbiochem) according to manufacturer's recommendations. Afterwards, lipids and salts were removed from the samples to improve resolution of 2D gels. Delipidation and salt depletion were accomplished by mixing one volume of plasma sample (that had been depleted from albumin and IgG) with four volumes of



methanol. After thoroughly mixing with the vortex, one volume of chloroform was added, the mixture was vortexed again and then, three volumes of water (HPLC grade) were incorporated. After centrifugation at 12,500 RPM for 5 min at 4°C, the upper phase was discarded. Three volumes of methanol were then added and the resultant mixture was vortexed again and centrifuged. The supernatant was discarded and the pellet containing precipitated plasma protein was air-dried for 5-10 min. About 0.7 mg of delipidated and salt-depleted plasma proteins were dissolved in 125 µl of sample solubilization solution (8.0 M urea, 50 mM DTT, 4% CHAPS, 0.2% carrier ampholytes and 0.0002% bromophenol blue). IPG strips (7cm long, pH range 4-7, BioRad, Hercules, CA, USA) were then passively rehydrated for 13h with the protein lysate. Isoelectrofocusing was carried out at 20 °C, using a PROTEAN IEF system (Bio-Rad). Focusing was started with a conditioning step of 250 V for 20 min, followed by a voltage ramping step to 4,000 V for 2 hours, and a final focusing step of 10,000 Vh. Thereafter, the strips were soaked in equilibration solution (50 mM Tris-HCl, pH 8.8, 6 M urea, 20% glycerol and 2% SDS) containing 20 mg/ml DTT for 10 min and then in the same solution containing 25 mg/ml iodoacetamide for an additional period of 10 min.

Second dimension was carried out in 12.5% polyacrylamide gels at a constant voltage of 200 V using a MINI-PROTEAN 3 Cell (BioRad). Proteins in 2-D gels were fixed with 40% methanol-10% acetic acid for 1 h at room temperature. Gels used for peptide identification and analysis were stained with colloidal Coomassie stain (BioSafe, Bio-Rad). Gel images were obtained using a GS-800 Calibrated Densitometer (Bio-Rad) and analyzed with the PDQuest 7.0 2-D analysis software (BioRad). To accurately compare spot quantities between gels, image spot quantities were normalized dividing the raw quantity of each spot in a gel by the total quantity of all the valid spots in that gel. Fluctuations in the protein expression levels among the control maps were monitored in densitometric analysis, and a coefficient of variation (CV) was generated for the mean value of each spot in order to better define changes in protein expression

due to CoQ supplementation. Only those changes that were statistically significant and that involved at least two-fold increases or half decreases of protein levels were considered in our study.

Protein spots of interest were manually excised from preparative gels, transferred to eppendorf tubes and automatically digested with trypsin according to standard protocols in a ProGest station (Genomic Solutions). Spots were destained twice for 30 min at 37°C with 200 mM ammonium bicarbonate/ 40% acetonitrile, and dehydrated for 5 minutes with pure acetonitrile. Then, spots were rehydrated with 25mM ammonium bicarbonate for 5 min and dehydrated again for 10 min with pure acetonitrile. After that, gel plugs were dried out for 4 h at room temperature. Then, 10  $\mu$ L of 12.5 ng/ $\mu$ L trypsin solution in 25 mM ammonium bicarbonate were added to each sample. Gels were allowed to rehydrate for 10 min at room temperature with the enzyme. The temperature was then adjusted to 37°C and digestion was carried out for 12 h. Finally, digestion volume was recovered and peptides were extracted with 10  $\mu$ L of 0,5% trifluoroacetic acid for 15 min. Peptides of each sample were analyzed in a 4700 Proteomics Station (Applied Biosystems, USA) in automatic mode. After drying, samples were analyzed in the  $m/z$  range 800 to 4000 Da with an accelerating voltage of 20 kV. Spectra were internally calibrated with peptides from trypsin autolysis. Proteins were identified by PMF and confirmed by MALDI-TOF/MS (APPLIED BIOSYSTEMS mod 4700). MASCOT searching engine (Matrixscience, UK) was used for protein identification over the database (MSDB). Only those proteins with MOWSE score above 88 ( $p$ , 0.00005) were accepted as valid identifications.

### 3. RESULTS

Animals supplemented on CoQ<sub>10</sub> reached a significantly higher mean (11.7%) and maximum (24%) lifespan (Quiles et al., 2004). Levels of CoQ<sub>9</sub> and CoQ<sub>10</sub> were quantified by HPLC in plasma samples obtained from rats fed for 6 or 24 months either with a PUFA<sub>n</sub>-6-alone or a PUFA<sub>n</sub>-6 plus CoQ<sub>10</sub> (0,7 mg/kg/ day) diet. As depicted in Figure 1, CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio was  $1.8 \pm 0.48$  in plasma of 6 months old rats fed on the PUFA<sub>n</sub>-6-alone diet, and this value increased to  $3.4 \pm 0.54$  at 24 months. Feeding animals with a CoQ<sub>10</sub>-supplemented diet produced a significant decrease of the plasma CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio both at 6 and 24 months.

Once we demonstrated that our lifelong supplementation schedule based on a low CoQ<sub>10</sub> concentration led to a good adaptation pattern, we studied how the CoQ<sub>10</sub>-enriched diet affected the levels of plasma proteins. Aging is associated with changes in the concentration of various serum proteins, and one of the most consistent findings is an age-dependent decrease in the level of albumin and increase in the content of some globulins (Chlebovská and Chlebovský, 1999; Kim et al., 2002). Thus, we first studied putative changes of plasma albumin levels as a function of aging and/or dietary CoQ<sub>10</sub>-supplementation. As depicted in Figure 2A and B, in our study we also observed a decrease of albumin levels in aged (24 months) compared to young (6 months) animals, which is consistent with previous observations. Although plasma albumin decreased with aging both in the control and in CoQ<sub>10</sub>-supplemented group, dietary administration of CoQ<sub>10</sub> increased significantly albumin at both ages in such a way that albumin levels at 24 months in the CoQ<sub>10</sub>-group were equivalent to those found at 6 months in the control group.

To obtain information about putative changes in less abundant plasma proteins that could be elicited by dietary CoQ<sub>10</sub>-supplementation, we carried out a proteomic study of rat plasma after depletion of albumin and IgG. At least four replicates were performed for all samples, similar protein spot patterns being obtained within each

sample type. Total numbers of protein spots identified in Coomassie blue-stained gels were: 244 (young rats, PUFA $n$ -6-alone diet), 218 (young rats, PUFA $n$ -6 plus CoQ $_{10}$  diet), 262 (old rats, PUFA $n$ -6-alone diet) and 232 (old rats, PUFA $n$ -6 plus CoQ $_{10}$  diet). Near 99% of all spots were matched on duplicate gels (the gels of the sample were produced, assayed, and processed in parallel), and the intensity of the same spot from different gels showed no significant change. A representative gel showing those proteins whose levels in plasma were altered in CoQ $_{10}$ -supplemented animals is depicted in Figure 3. Only proteins that increased or decreased by a factor of at least two were considered in this study. This gel is annotated to show the location of spots excised and identified by peptide mass fingerprinting and the proteins identified are listed in Table I. The predicted molecular weight and pI of protein spots were used to corroborate protein identifications. Details of 2D gels showing those proteins whose levels in plasma from CoQ $_{10}$ -supplemented animals were significantly ( $p < 0.05$ ) altered compared with their corresponding controls are depicted in Figure 4.

Preprohaptoglobin was significantly decreased (3-5 times) in the CoQ $_{10}$  group at 6 months and hemopexin was significantly decreased (about 2-fold) at both 6 and 24 months. Type II peroxiredoxin levels decreased (3-4 fold) at 24 months in the CoQ $_{10}$ -group. Plasma apolipoprotein H ( $\beta_2$ -glycoprotein I,  $\beta_2$ GPI) was decreased in the CoQ $_{10}$ -group at both 6 and 24 months. Levels of  $\beta_2$ GPI were halved at 6 months in animals fed with the CoQ $_{10}$ -diet, and the protein was almost undetectable at 24 months. Levels of another apolipoprotein, Apo A-I were also altered by dietary CoQ $_{10}$ , although in this case the protein was increased in the CoQ $_{10}$ -group (two-fold at 24 months). Fibrinogen  $\gamma$ -chain precursor was decreased three times by dietary CoQ $_{10}$  at 6 months, although no significant effect was observed at 24 months. Inter-alpha-inhibitor H4P heavy chain was halved at 6 and 24 months in the CoQ $_{10}$ -group. Two protein spots that were identified as inter-alpha-inhibitor H4P heavy chain fragments were also consistently found in the plasma proteome of control animals, and these spots were strongly decreased (more than ten times) in the CoQ $_{10}$ -group at 6 months (see Figures 3 and 4). Levels of alpha-1-

antitrypsin precursor were halved by CoQ<sub>10</sub>-supplementation at 24 months, although no significant effects were observed at 6 months. Serine protease inhibitor 3 was increased more than ten times at 6 months in the CoQ<sub>10</sub>-group, although no significant alterations were observed at 24 months. Finally, we observed changes elicited by dietary CoQ<sub>10</sub> in vitamin D-binding protein (which increased at 6 months in the CoQ<sub>10</sub> group) and fetuin-like protein (which was strongly decreased by CoQ<sub>10</sub>-supplementation at 6 months).

#### 4. DISCUSSION

Rat plasma and tissues contain both CoQ<sub>9</sub> and CoQ<sub>10</sub> as the endogenous CoQ forms (Turunen et al., 2004). Thus, we used the decrease of CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio as an estimation of supplementation with dietary CoQ<sub>10</sub>. This ratio may be of importance for aging studies because longevity correlates positively with CoQ<sub>10</sub> levels, and negatively with CoQ<sub>9</sub> levels in different mammal species (Lass et al., 1997). Plasma CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio was significantly decreased in the CoQ<sub>10</sub>-group indicating an effective supplementation with exogenous CoQ<sub>10</sub> regardless of age. This is very important because any putative benefit of dietary supplementation with CoQ<sub>10</sub> might be maintained through the whole life (Quiles et al., 2004). According to our studies (Gómez-Díaz et al., 2003; Bello et al., 2005; this work) and those from others (Sugiyama et al., 1995; Forsmark-Andrée et al., 1997; Matthews et al., 1998; Lönnrot et al., 1998; Kwong et al., 2002), plasma and liver show a better adaptation pattern to dietary CoQ<sub>10</sub> than organs like brain or heart. In accordance, significant decrease in CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio was also found in liver samples obtained from the same animals (Bello et al., 2005). Calorie restriction also decreases CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio in rats and mice liver (de Cabo et al., 2004; López-Lluch et al., 2005).

The actual concentration of plasma/serum proteins is a result of metabolic processes, maintaining homeostasis. With advancing age a progressive weakening of these factors occurs which results in the unfavourable effect on the whole protein turnover with subsequent changes in the concentration of individual proteins (Chlebovská and Chlebovský, 1999). Lower levels of serum albumin are associated with increased risk of all-cause and cardiovascular mortality as well with coronary heart disease and stroke incidence in humans. In addition, albumin is a negative acute-phase reactant (Shaper et al., 2004). It has been proposed that the aging-related decrease of serum albumin in the rat may be partially responsible for decreased serum thiol levels and a shifted redox serum balance towards oxidation during aging (Chlebovská and

Chlebovský, 1999). In addition to its antioxidant role inhibiting lipid peroxidation and regenerating  $\alpha$ -tocopherol in serum lipoproteins (Stocker et al., 1991; Mohr et al., 1992), dietary CoQ<sub>10</sub> supplementation might further enhance antioxidant capacity of blood plasma by increasing albumin levels.

Besides albumin, proteins which levels were altered as a consequence of dietary CoQ<sub>10</sub> supplementation fall into the following functional groups: i. heme metabolism: preprohaptoglobin and hemopexin; antioxidant protection: peroxiredoxin 2 (and preprohaptoglobin and hemopexin as well); ii. lipid metabolism and transport: apolipoprotein H and apolipoprotein A1; iii. blood coagulation: fibrinogen  $\gamma$ -chain precursor; iv. acute-phase proteins: inter-alpha-inhibitor H4P, serine protease inhibitor 3 (and preprohaptoglobin and hemopexin as well); and osteogenesis: vitamin D-binding protein and fetuin-like protein.

Preprohaptoglobin was decreased in the CoQ<sub>10</sub> group at 6 months, and hemopexin was decreased at both 6 and 24 months. Haptoglobin is a serum protein that binds hemoglobin and allows its metabolization in the liver. Haptoglobin also functions as an antioxidant because it prevents oxidative damage mediated by free haemoglobin (Melamed-Frank et al., 2001). Haptoglobin has been shown to be a potential marker of cancer (Thadikkaran et al., 2005). Hemopexin is a plasma glycoprotein with a high binding affinity to heme, which is then transported to the liver. The presence of heme in plasma is due to oxidation of haemoglobin, which is released during the enucleation of erythroblasts or in states of intramedullary or intravascular haemolysis. In humans, haemolysis is associated with several pathologic conditions such as reperfusion injury and/or ischemia. Hemopexin also inhibits some of the oxidative reactions catalyzed by free heme and may protect tissues from oxidation under conditions in which heme release occurs (Barnard et al., 1993; Tolosano et al., 1999). Previous studies have documented that levels of these two proteins of the acute phase increase in rat serum with aging, and their levels were further increased in animals whose aging was

accelerated by irradiation (Chlebovská and Chlebovský, 1999). Interestingly, these aging-related changes were attenuated by dietary CoQ<sub>10</sub>.

Type II peroxiredoxin was significantly decreased at 24 months in the CoQ<sub>10</sub>-group. Peroxiredoxins are a family of thiol-specific peroxidases found in all species that can reduce a wide range of peroxides, including toxic phospholipid hydroperoxides, thus playing a major role in antioxidant protection (Wong et al., 2004; Tölle et al., 2005). Although peroxiredoxin levels decrease with aging in rodents, which could be indicative of compromised antioxidant protection with aging (Cho et al., 2003), this protein is induced by oxidative stress conditions provoked by alimentary vitamin E deficiency (Tölle et al., 2005). Thus, it is plausible to interpret that decreased levels of peroxiredoxin in plasma obtained from CoQ<sub>10</sub>-supplemented animals (as well as decreased hemopexin and preprohaptoglobin, see above) may be indicative of a better antioxidant status compared to non supplemented animals.

$\beta_2$ GPI was decreased in the CoQ<sub>10</sub>-group at both 6 and 24 months.  $\beta_2$ GPI is the main target antigen for antiphospholipid antibodies in patients with antiphospholipid syndrome, and has been shown to regulate the activation of plasminogen. Although the exact physiological role of  $\beta_2$ GPI is not well understood (Lopez-Lira et al., 2006), it is known that  $\beta_2$ GPI forms complexes with oxidized LDL, which represent a common metabolic product relevant to atherogenesis and a risk factor or an indirect but significant contributor for atherothrombotic complications in autoimmune patients (Matsuura et al., 2006). Levels of another apolipoprotein, Apo A-I were also altered by dietary CoQ<sub>10</sub>, although in this case the protein was increased in the CoQ<sub>10</sub>-group (two-fold at 24 months). It is important to note that high concentrations of Apo A-I are associated with lower mortality and myocardial infarction five years after coronary artery bypass graft surgery in humans (Anderson, 2005). Interestingly, dietary supplementation with  $\alpha$ -tocopherol, another lipophilic antioxidant as CoQ<sub>10</sub>, also elevates plasma Apo A-I levels in healthy humans (Aldred et al., 2006).



Levels of fibrinogen  $\gamma$ -chain precursor were decreased by dietary CoQ<sub>10</sub> at 6 months, although no significant effect was observed at 24 months. Fibrinogen is strongly, consistently, and independently related to cardiovascular risk (Koenig, 2003). Interestingly, many experimental studies have indicated that exogenous CoQ<sub>10</sub> treatment may be beneficial in cardiovascular pathologies (Thomas et al., 2001), and a relation exists between low plasma CoQ<sub>10</sub> concentration and coronary artery disease in humans (Yalcin et al., 2004). Changes in plasma proteins elicited by dietary CoQ<sub>10</sub> could explain, at least partially, these beneficial effects on the cardiovascular system.

Besides hemopexin and preprohaptoglobin (see above), other acute phase proteins were also decreased by dietary CoQ<sub>10</sub>-supplementation. Inter-alpha-inhibitor H4P belongs to a family of plasma protease inhibitors of hepatic origin, collectively termed as the inter-alpha-inhibitor family. Levels of inter-alpha-inhibitor H4P increase significantly under acute inflammatory conditions (Daveau et al., 1998). Alpha-1-antitrypsin precursor is also considerably increased during the acute-phase response (Sandford et al., 1999). Although inter-alpha-inhibitor H4P heavy chain and alpha-1-antitrypsin precursor were decreased significantly by CoQ<sub>10</sub>-supplementation, serine protease inhibitor 3, another acute-phase protein, was increased more than ten times at 6 months in the CoQ<sub>10</sub>-group, although no significant alterations were observed at 24 months. Changes in the levels of acute-phase proteins due to CoQ<sub>10</sub>-supplementation indicate that dietary CoQ<sub>10</sub> may play an important role in the regulation of inflammation. In accordance, a previous study has documented that co-supplementation with vitamin E and CoQ<sub>10</sub> reduces circulating markers of inflammation in baboons (Wang et al., 2004).

Finally, we observed changes elicited by dietary CoQ<sub>10</sub> in two plasma proteins involved in bone metabolism, vitamin D-binding protein and fetuin. Vitamin D-binding protein is an abundant serum glycoprotein which transports vitamin D sterols and binds to monomeric actin (Cooke, 1986). On the other hand, fetuin is a serum protein which inhibits osteogenesis and may promote artery calcification (Binkert et al., 1999; Price et

al., 2004). In addition, fetuin concentrations are strongly associated with metabolic syndrome and an atherogenic lipid profile in humans (Ix et al., 2006). The fact that dietary CoQ<sub>10</sub> increased vitamin D-binding protein and decreased fetuin-like protein might indicate that CoQ<sub>10</sub> status might also influence osteogenesis. It is noteworthy that rats fed lifelong on the PUFA $\alpha$ -6 plus CoQ<sub>10</sub> diet showed increased bone mineral density compared to their control counterparts (Ochoa et al., 2005). This effect could be also related to the role of serum CoQ<sub>10</sub> as antioxidant because it has been reported that oxidative stress levels are negatively associated with bone mineral density, and antioxidant levels are lower in osteoporosis patients (Basu et al., 2001; Maggio et al., 2003).

Overall, our proteomic study strongly supports that increase of lifespan provided by a CoQ<sub>10</sub>-enriched diet in the rat is related to decreased oxidative stress, and less cardiovascular risk and inflammation during aging.

#### **ACKNOWLEDGEMENTS**

Supported by Grant No. BFU2005-00137/BMC (Spanish Ministerio de Educación y Ciencia), and CVI-276 (Junta de Andalucía). M.S. and C. G.-D. were supported by CVI-276.

**REFERENCES**

Aldred, S., Sozzi, T., Mudway, I., Grant, M.M., Neubert, H., Kelly, F.J., Griffiths, H.R., 2006. Alpha tocopherol supplementation elevates plasma apolipoprotein A1 isoforms in normal healthy subjects. *Proteomics* 6, 1695-1703.

Anderson, L., 2005. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J. Physiol.* 563.1, 23-60.

Barja, G., 2004. Free radicals and aging. *Trends Neurosci.* 27, 595-600.

Barja, G., Herrero, A., 2000. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J.* 14, 312-318.

Barnard, M.L., Muller-Eberhard, U., Turrens, J.F., 1993. Protective role of hemopexin on heme-dependent lung oxidative stress. *Biochem. Biophys. Res. Commun.* 192, 82-87.

Basu, S., Michaelsson, K., Olofsson, H., Johansson, S., Melhus, H., 2001. Association between oxidative stress and bone mineral density. *Biochem. Biophys. Res. Commun.* 288, 275-279.

Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V.V., Allard, J.S., López-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S., Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W., Spencer, R.G., Lakatta, E.G., Le Couteur, D., Shaw, R.J., Navas, P., Puigserver, P., Ingram, D.K., de Cabo, R., Sinclair, D.A., 2006. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337-342.

Bello, R.I., Gómez-Díaz, C., Burón, M.I., Alcaín, F.J., Navas, P., Villalba, J.M., 2005. Enhanced anti-oxidant protection of liver membranes in long-lived rats fed on a coenzyme Q<sub>10</sub>-supplemented diet. *Exp. Gerontol.* 40, 694-706.

Binkert, C., Demetriou, M., Sukhu, B., Szweras, M., Tenenbaum, H.C., Dennis, J.W., 1999. Regulation of osteogenesis by fetuin. *J. Biol. Chem.* 274, 28514-28520.

Chlebovská, K., Chlebovský, O., 1999. Favourable effect of K, Mg aspartate on serum proteins in aging rats. *Mech. Ageing Dev.* 108, 127-138.

Cho, Y.M., Bae, S.H., Choi, B.K., Cho, S.Y., Song, C.W., Yoo, J.K., Paik, Y.K., 2003. Differential expression of the liver proteome in senescence accelerated mice. *Proteomics* 3, 1883-1894.

Cooke, N.E., 1986. Rat vitamin D binding protein. Determination of the full-length primary structure from cloned cDNA. *J. Biol. Chem.* 261, 3441-3450.

Daveau, M., Jean, L., Soury, E., Olivier, E., Masson, S., Lyoumi, S., Chan, P., Hiron, M., Lebreton, J.P., Husson, A., Jegou, S., Vaudry, H., Salier, J.P., 1998. Hepatic and extra-hepatic transcription of inter-alpha-inhibitor family genes under normal or acute inflammatory conditions in rat. *Arch. Biochem. Biophys.* 350, 315-323.

de Cabo, R., Cabello, R., Rios, M., López-Lluch, G., Ingram, D.K., Lane, M.A., Navas, P., 2004. Calorie restriction attenuates age-related alterations in the plasma membrane antioxidant system in rat liver. *Exp. Gerontol.* 39: 297-304.

Echan, L.A., Tang, H.-Y., Ali-Khan, N., Lee, K., Speicher, D.W., 2005. Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma. *Proteomics* 5, 3292-3303.

Finkel, T., 2003. A toast to long life. *Nature* 425, 132-133.

- Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of aging. *Nature* 408, 239-247.
- Forsmark-Andrée, P., Lee, C.P., Dallner, G., Ernster, L., 1997. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radic. Biol. Med.* 22, 391-400.
- Gómez-Díaz, C., Burón, M.I., Alcaín, F.J., González-Ojeda, R., González-Reyes, J.A., Bello, R.I., Hermán, M.D., Navas, P., Villalba, J.M., 2003. Effect of dietary coenzyme Q and fatty acids on the antioxidant status of rat tissues. *Protoplasma* 221, 11-17.
- Harman, D., 2003. The free radical theory of aging. *Antioxid. Redox Signal.* 5, 557-561.
- Hekimi, S., Guarente, L., 2003. Genetics and the specificity of the aging process. *Science* 299, 1351-1354.
- Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 425, 191-196.
- Ix, J.H., Shlipak, M.G., Brandenburg, V.M., Ali, S., Ketteler, M., Whooley, M.A., 2006. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. *Circulation* 113, 1760-1767.
- Keightley, J.A., Shang, L., Kinter, M., 2004. Proteomic analysis of oxidative stress-resistant cells: a specific role for aldose reductase overexpression in cytoprotection. *Mol. Cell. Proteomics* 3, 167-175.
- Kim, J.W., No, J.K., Ikeno, Y., Yu, B.P., Choi, J.S., Yokozawa, T., Chung, H.Y., 2002. Age-related changes in redox status of rat serum. *Arch. Gerontol. Geriatr.* 34, 9-17.

Kirkwood, T.B.L., Austad, S.N., 2000. Why do we age? *Nature* 408, 233-238.

Koenig, W., 2003. Fibrin(ogen) in cardiovascular disease: an update. *Thromb. Haemost.* 89, 601-609.

Kwong, L.K., Kamzalov, S., Rebrin, I., Bayne, A.C., Jana, C.K., Morris, P., Forster, M.J. Sohal, R.S., 2002. Effects of coenzyme Q<sub>10</sub> administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radic. Biol. Med.*, 33, 627-638.

Lass, A., Agarwal, S., Sohal, R.S., 1997. Mitochondrial ubiquinone homologues, superoxide radical generation, and longevity in different mammalian species. *J. Biol. Chem.* 272, 19199–19204.

Lönnrot, K., Hola, P., Lagerstedt, A., Huhtala, H., Alho, H., 1998. The effects of lifelong ubiquinone Q<sub>10</sub> supplementation on the Q<sub>9</sub> and Q<sub>10</sub> tissue concentrations and life span of male rats and mice. *Biochem. Mol. Biol. Int.* 44, 727-737.

Lopez-Lira, F., Rosales-León, L., Martínez, V.M., Ruíz-Ordaz, B.H., 2006. The role of  $\beta_2$ -glycoprotein I ( $\beta_2$ GPI) in the activation of plasminogen. *Biochim. Biophys. Acta* 1764, 815-823.

López-Lluch, G., Ríos, M., Lane, M.A., Navas, P., De Cabo, R., 2005. Mouse liver plasma membrane redox system activity is altered by aging and modulated by calorie restriction. *AGE* 27, 153-160.

Maggio, D., Barabani, M., Pierandrei, M., Polidori, M.C., Catani, M., Mecocci, P., Senin, U., Pacifici, R., Cherubini, A., 2003. Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. *J. Clin. Endocrinol. Metab.* 88, 1523-1527.

Matsuura, E., Kobayashi, K., Tabuchi, M., López, L.R., 2006. Oxidative modification of low-density lipoprotein and immune regulation of atherosclerosis. *Prog. Lipid Res.* 45, 466-486.

Matthews, R.T., Yang, L., Browne, S., Baik, M., Beal, M.F., 1998. Coenzyme Q<sub>10</sub> administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc. Natl. Acad. Sci. USA* 95, 8892-8897.

Melamed-Frank, M., Lache, O., Enav, E.I., Szafrank, T., Levy, N.S., Ricklis, R.M., Levy, A.P., 2001. Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* 98, 3693-3698.

Mohr, D., Bowry, V.W., Stocker, R., 1992. Dietary supplementation with coenzyme Q<sub>10</sub> results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation. *Biochim. Biophys. Acta* 1126, 247-254.

Ochoa, J.J., Quiles, J.L., Huertas, J.R., Mataix, J., 2005. Coenzyme Q<sub>10</sub> protects from aging-related oxidative stress and improves mitochondrial function in heart of rats fed a polyunsaturated fatty acid (PUFA)-rich diet. *J. Gerontol.* 60, 970-975.

Ochoa, J.J., Quiles, J.L., Planells, E., López-Frías, M., Huertas, J.R., Llamas, J.M., and Mataix, J. (2005) Lifelong supplementation with coenzyme Q protects from bone mineral density loss in rats during aging. *Proceedings of the 4<sup>th</sup> Conference of the International Coenzyme Q<sub>10</sub> Association (Los Angeles, CA)*, pp. 149-150.

Price, P.A., Williamson, M.K., Nguyen, T.M.T., Than, T.N., 2004. Serum levels of the fetuin-mineral complex correlate with artery calcification in the rat. *J. Biol. Chem.* 279, 1594-1600.

Quiles, J.L., Ochoa, J.J., Huertas, J.R., Mataix, J., 2004. Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increased lifespan in rats fed on a PUFA-rich diet. *Exp. Gerontol.* 39, 189-194.

Sandford, A.J., Chagani, T., Spinelli, J.J., Paré, P.D., 1999. Alpha1-antitrypsin genotypes and the acute-phase response to open heart surgery. *Am. J. Respir. Crit. Care Med.* 159, 1624-1628.

Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., Oskun, P.E., Ladiges, W., Wolf, N., Remmen, H.V., Wallace, D.C., Rabinovitch, P.S., 2005. Extension of murine lifespan by overexpression of catalase targeted to mitochondria. *Science* 308, 1909-1911.

Shaper, A.G., Wannamethee, S.G., Whincup, P.H., 2004. Serum albumin and risk of stroke, coronary heart disease, and mortality: the role of cigarette smoking. *J. Clin. Epidemiol.* 57, 195-202.

Sohal, R.S., 2002. Oxidative stress hypothesis of aging. *Free Radic. Biol. Med.* 33, 573-574.

Sohal, R.S., Mockett, R.J., Orr, W.C., 2002. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic. Biol. Med.* 33, 575-586.

Stocker, R., Bowry, V.W., Frei, B., 1991. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does  $\alpha$ -tocopherol. *Proc. Natl. Acad. Sci. USA* 88, 1646-1650.

Stoscheck, C.M., 1990. Quantitation of protein. *Methods Enzymol.* 182, 50-68.

Sugiyama, S., Yamada, K., Ozawa, T., 1995. Preservation of mitochondrial respiratory function by coenzyme Q10 in aged rat skeletal muscle. *Biochem. Mol. Biol. Int.* 37, 1111-1120.



Thadikaran, L., Siegenthaler, M.A., Crettaz, D., Queloz, P.-A., Schneider, P., Tissot, J.D., 2005. Recent advances in blood-related proteomics. *Proteomics* 5, 3019-3034.

Thomas, S.R., Leichtweis, S.B., Pettersson, K., Croft, K.D., Mori, T.A., Brown, A.J., Stocker, R., 2001. Dietary cosupplementation with vitamin E and coenzyme Q<sub>10</sub> inhibits atherosclerosis in apolipoprotein E gene knockout mice. *Arterioscler. Thromb. Vasc. Biol.* 21, 585-593.

Tölle, A., Schlame, M., Charlier, N., Guthmann, F., Rustow, B., 2005. Vitamin E differentially regulates the expression of peroxiredoxin-1 and -6 in alveolar type II cells. *Free Radic. Biol. Med.* 38, 1401-1408.

Tolosano, E., Hirsch, E., Patrucco, E., Camaschella, C., Navone, R., Silengo, L., Altruda, F., 1999. Defective recovery and severe renal damage after acute hemolysis in hemopexin-deficient mice. *Blood* 94, 3906-3914.

Turunen, M., Olsson, J., Dallner, G., 2004. Metabolism and function of coenzyme Q. *Biochim. Biophys. Acta* 1660, 171-199.

Wallace, D.C., 1992. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 256, 628-632.

Wang, X.L., Rainwater, D.L., Mahaney, M.C., Stocker, R., 2004. Cosupplementation with vitamin E and coenzyme Q<sub>10</sub> reduces circulating markers of inflammation in baboons. *Am. J. Clin. Nutr.* 80, 649-655.

Wong, C.M., Siu, K.L., Jin, D.Y., 2004. Peroxiredoxin-null yeast cells are hypersensitive to oxidative stress and are genomically unstable. *J. Biol. Chem.* 279, 23207-23213.

Yalcin, A., Kilinc, E., Sagcan, A., Kultursay, H., 2004. Coenzyme Q<sub>10</sub> concentrations in coronary artery disease. *Clin. Biochem.* 37, 706-709.

## LEGENDS FOR FIGURES

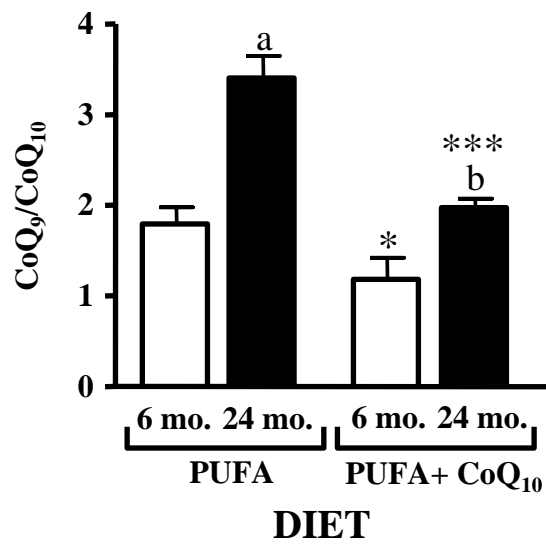
**Figure 1.** CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio in plasma. Rats were fed for 6 or 24 months with a basal semisynthetic PUFA $n$ -6-rich diet, either supplemented or not with CoQ<sub>10</sub> (intake of 0.7 mg/kg/day). Lipids were extracted from plasma and CoQ<sub>10</sub> and CoQ<sub>9</sub> quantified by HPLC. Values are means with standard errors (n=8). Significant differences between diets for a given feeding period are indicated with asterisks (\*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ). Statistically significant differences between feeding periods for a given diet are also indicated (a, statistically significant differences with 6 months,  $P < 0.05$ ; b, statistically significant differences with 6 months,  $P < 0.001$ ). We demonstrate that our dietary protocol increases CoQ<sub>10</sub> levels (measured as a decrease of CoQ<sub>9</sub>/CoQ<sub>10</sub> ratio in plasma) both at 6 and 24 months.

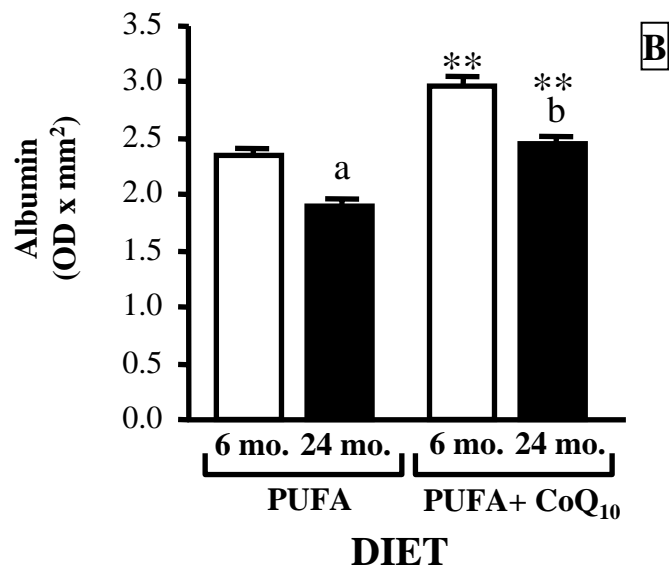
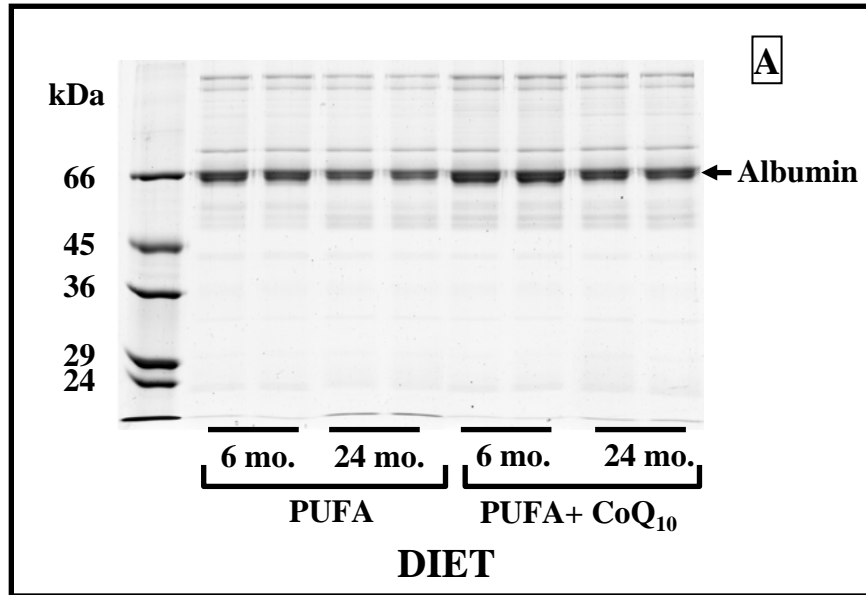
**Figure 2.** Effect of dietary CoQ<sub>10</sub> supplement on albumin levels. Rats were fed for 6 or 24 months as described in the legend of figure 1 and equal amounts of plasma proteins (3  $\mu$ g) were separated by SDS-PAGE (10% acrylamide). Gels were stained with colloidal Coomassie stain and albumin was then quantified by densitometric analysis of the 66 kDa band. A) Two representative samples from each condition are depicted. B) Albumin quantification by densitometry (OD x mm<sup>2</sup>). Values in (B) are means with standard errors (n=8). Significant differences between diets for a given feeding period are denoted with asterisks (\*\*,  $P < 0.01$ ). Statistically significant differences between feeding periods for a given diet are also indicated (a, statistically significant differences with 6 months,  $P < 0.05$ ; b, statistically significant differences with 6 months,  $P < 0.05$ ). Our results demonstrate that albumin levels decrease with aging, whereas dietary CoQ<sub>10</sub> supplement increases albumin both at 6 and 24 months.

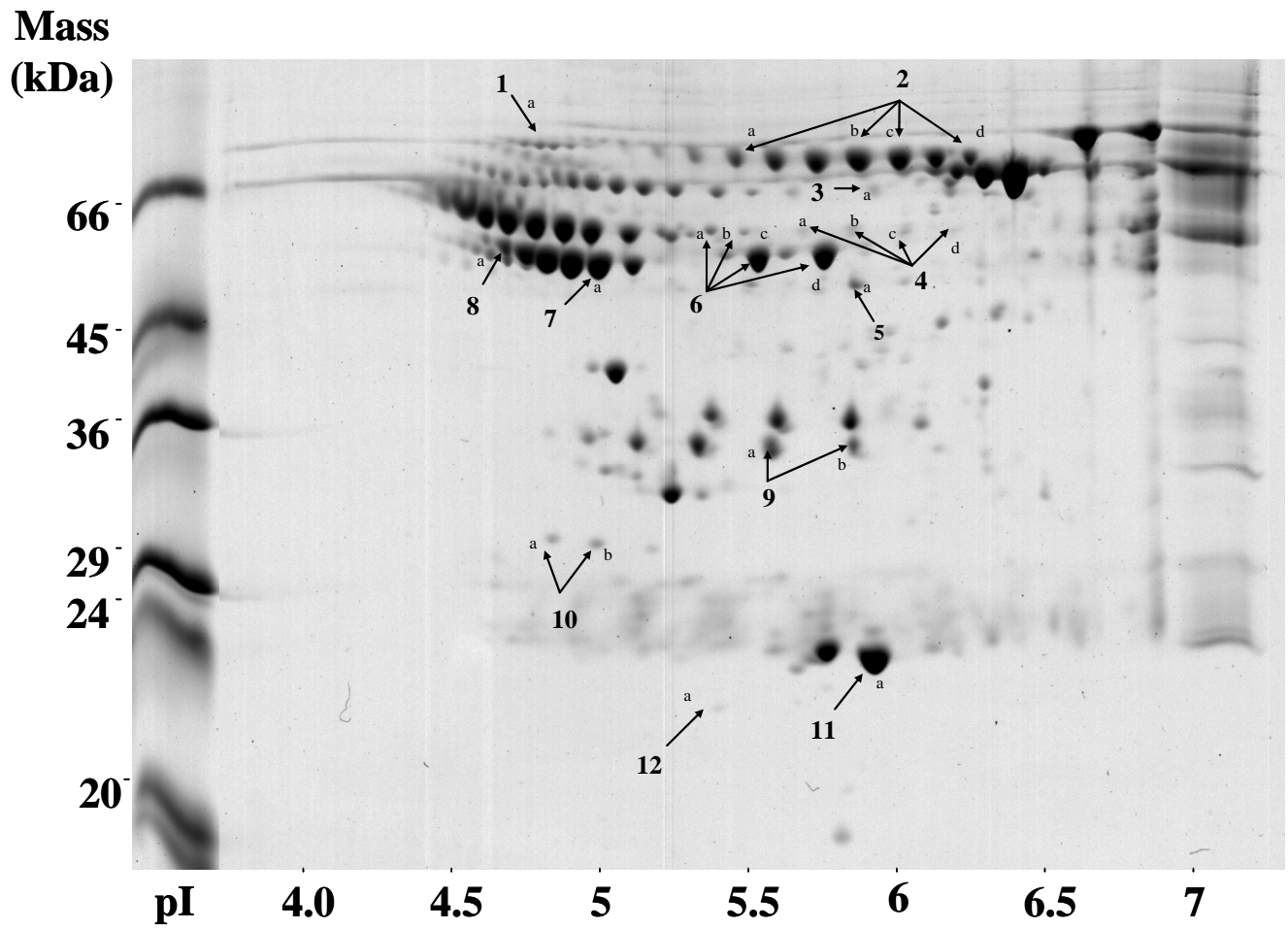
**Figure 3.** A representative 2D gel electrophoresis image showing the most abundant proteins of albumin and IgG-depleted rat plasma. The scanned image of the coomassie-stained gel was used to detect and compare protein spots. Those

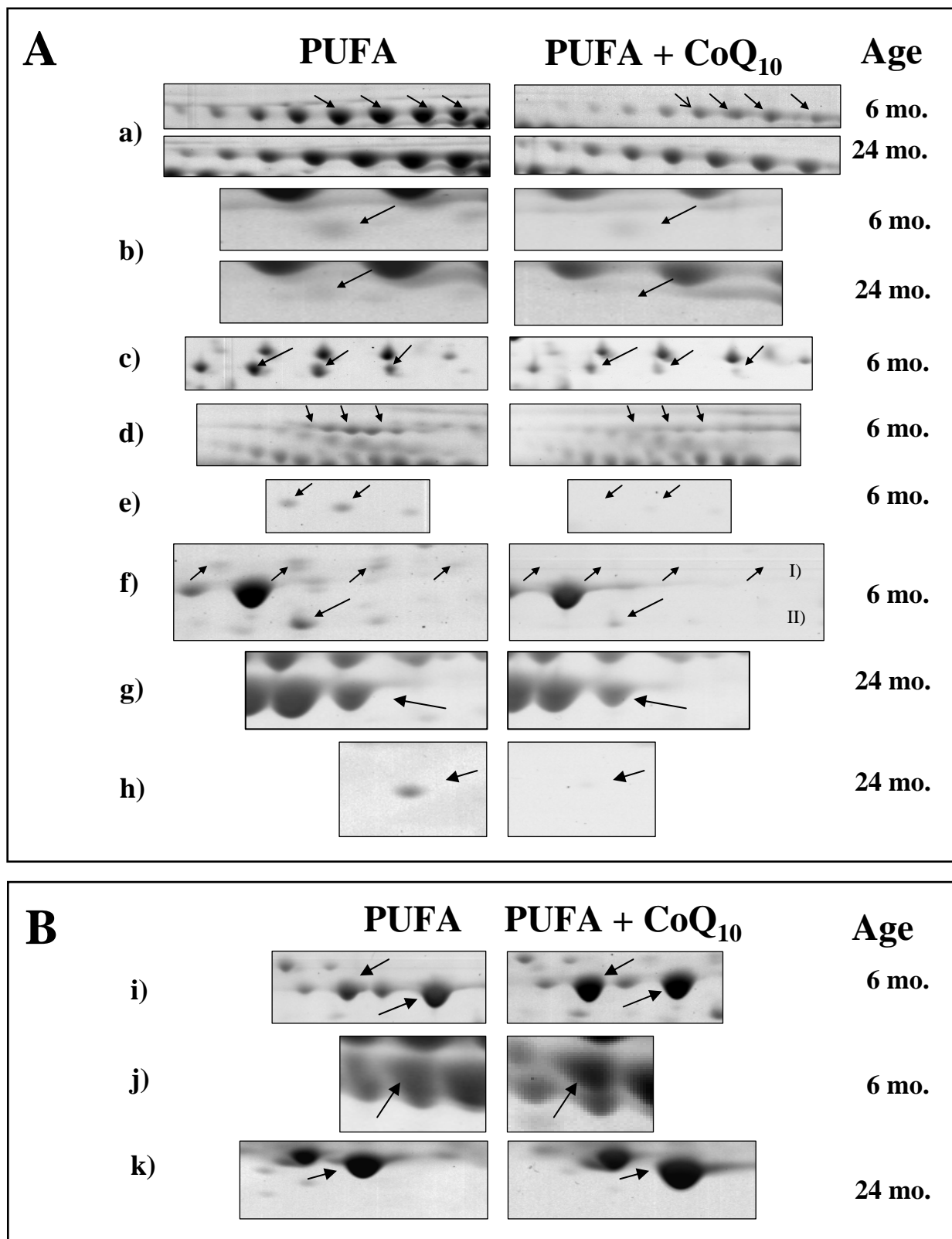
proteins whose levels were significantly altered in rats fed on a CoQ<sub>10</sub>-enriched diet are numbered and indicated by arrows. Letters indicate different isoforms that were identified by mass spectrometry. Gradient of pH (first dimension) are indicated at the bottom of the gel, and the positions of molecular mass standards (in kilodaltons) are shown in the left. Protein spots of different isoforms that were identified by mass spectrometry are denoted with letters. (1) inter-alpha-inhibitor H4P heavy chain, (2) hemopexin precursor, (3) apolipoprotein H precursor, (4) fetuin-like protein, (5) fibrinogen  $\gamma$ -chain precursor, (6) vitamin D-binding protein precursor, (7) alpha-1-antitrypsin precursor, (8) serine protease inhibitor 3, (9) preprohaptoglobin precursor, (10) inter-alpha-inhibitor H4P heavy chain (fragments), (11) Apo A-I, (12) type II peroxiredoxin.

**Figure 4.** Close-up sections of protein spots of proteins whose levels are significantly altered in plasma of rats fed on a CoQ<sub>10</sub>-enriched diet. The animal age when changes were observed is denoted at the right. **A)** This panel depicts those proteins whose levels were decreased in the CoQ<sub>10</sub>-group. a) hemopexin precursor, b) apolipoprotein H precursor, c) preprohaptoglobin precursor, d) inter-alpha-inhibitor H4P heavy chain, e) inter-alpha-inhibitor H4P heavy chain (fragments), f-I) fetuin-like protein, f-II) fibrinogen  $\gamma$ -chain precursor, g) alpha-1-antitrypsin precursor, h) type II peroxiredoxin. **B)** This panel depicts those proteins whose levels were increased in the CoQ<sub>10</sub>-group. i) vitamin D-binding protein precursor, j) serine protease inhibitor 3, k) Apo A-I.









**Table I.** Proteins with altered levels in plasma obtained from long-lived rats fed on a CoQ<sub>10</sub>-enriched diet.

Function of the protein	Protein name	Spot	Accession n° (MSDB)	Sequence coverage (%)	Score	No. of matched peptides	Mr ( Da)	Aterations after coenzyme Q supplementation
<b>Heme metabolism and antioxidant protection. Acute-phase proteins</b>	Hemopexin precursor	2,a)	OQRT	43	212	20	51999	Decreases at 6 and 24 months
	Hemopexin precursor	2,b)	OQRT	39	197	20	51999	Decreases at 6 and 24 months
	Hemopexin precursor	2,c)	OQRT	36	119	16	51999	Decreases at 6 and 24 months
	Hemopexin precursor	2,d)	OQRT	45	349	22	51999	Decreases at 6 and 24 months
	Preprohaptoglobin precursor	9,a)	AAA41349	51	264	15	30428	Decreases at 6 months
	Preprohaptoglobin precursor	9,b)	AAA41349	49	241	15	30428	Decreases at 6 months
<b>Ostogenesis</b>	Fetuin-like protein IRL685	4,a)	CAB62543	43	236	14	42361	Decreases at 6 months
	Fetuin-like protein IRL685	4,b)	CAB62543	48	321	17	42361	Decreases at 6 months
	Fetuin-like protein IRL685	4,c)	CAB62543	43	281	16	42361	Decreases at 6 months
	Fetuin-like protein IRL685	4,d)	CAB62543	47	148	15	42361	Decreases at 6 months
	Vitamin D-binding protein precursor	6,a)	AAA41082	54	423	22	55078	Increases at 6 months
	Vitamin D-binding protein precursor	6,b)	AAA41082	34	185	14	55078	Increases at 6 months
	Vitamin D-binding protein precursor	6,c)	AAA41082	59	373	25	55078	Increases at 6 months
	Vitamin D-binding protein precursor	6,d)	AAA41082	55	245	21	55079	Increases at 6 months
<b>Acute-phase proteins</b>	Alpha-1-antitrypsin precursor	7,a)	AAA40788	42	191	16	45978	Decreases at 24 months
	Serine protease inhibitor 3	8,a)	S11320	35	185	16	45639	Increases at 6 months
	Inter-alpha-inhibitor H4P heavy chain	1,a)	JC5953	22	263	23	103884	Decreases at 6 and 24 months
	Inter-alpha-inhibitor H4P heavy chain (fragment)	10,a)	JC5953	15	125	10	103884	Decreases at 6 months
	Inter-alpha-inhibitor H4P heavy chain (fragment)	10,b)	JC5953	14	162	11	103884	Decreases at 6 months
<b>Lipid metabolism and transport</b>	Apolipoprotein H precursor (B2GPI)	3,a)	P26644	39	118	14	34315*	Decreases at 6 and 24 months
	Apolipoprotein A-I precursor	11,a)	CAA25224	65	374	21	30100	Increases at 24 months
<b>Blood coagulation</b>	Fibrinogen gamma chain precursor	5,a)	CAA29289	48	180	16	50247	Decreases at 6 months
<b>Antioxidant protection</b>	Peroxirredoxin 2 (Thioredoxin peroxidase 1)	12,a)	Q9CWJ4	27	244	6	21968	Decreases at 24 months