Eur J Nutr (2010) 49:181–187
DOI 10.1007/s00394-009-0064-4

ORIGINAL CONTRIBUTION

Effect of beer consumption on levels of complex I and complex IV liver and heart mitochondrial enzymes and coenzymes Q9 and Q10 in adriamycin-treated rats

Victoria Valls-Belles · Carmen Torres · Pilar Muñiz · Pilar Codoñer-Franch

Received: 21 April 2009 / Accepted: 30 September 2009 / Published online: 20 October 2009

Abstract
Background There is increasing evidence indicating that the dietary intake of food with high antioxidant capacity may protect mitochondria from damage and exert positive effects on different pathogenic processes.
Aim of the study The present study was designed to evaluate the possible protective effect of alcohol-free beer intake on chain components dysfunction of liver and heart mitochondria, and to compare with the effect of alcohol beer intake.
Methods The study was carried out in rat heart and liver mitochondria by inducing with Adriamycin the dysfunction of the respiratory chain. Heart and liver mitochondria were isolated from rats and subjected to oxidative stress with two doses of Adriamycin (5 mg/Kg) 7 days from the beginning of consumption of both alcohol-free and alcohol beer during 31 days. Complexes I and IV and the levels of coenzymes Q_9 and Q_{10} were evaluated and compared with a control group.
Results Liver and heart mitochondria isolated from rats treated with Adryamicin showed a decrease in levels of complex I and complex IV enzymatic activity and in levels of coenzymes Q_9 and Q_{10}. Beer intake for itself does not affect any of the studied parameters. Therefore, the consumption of both alcohol and alcohol-free beer by rats treated with Adriamycin prevents the inhibition of enzymatic activities of complexes I and IV and the oxidation of coenzymes Q_9 and Q_{10} in rat heart and liver mitochondria.
Conclusions These results indicate that alcohol-free beer prevents adriamycin-induced damage to mitochondrial chain components and, therefore, helps to prevent mitochondrial dysfunction.

Keywords Alcohol-free beer · Adriamycin · Mitochondrial enzymes · Coenzymes Q

Introduction

Mitochondria play a central role in both cell life and death. These organelles are essential for the production of ATP via oxidation, phosphorylation and regulation of intracellular Ca^{+2} homeostasis. Alterations in mitochondrial complexes involved in energy conservation result in defective electron transfer and oxidative phosphorylation [9, 26]. Production of free radicals by the mitochondrial transport chain is one of the causes of mitochondrial damage, which is involved in the pathogenesis of different diseases. The use of antioxidants present in foods could play an important role in the prevention of this mitochondrial damage [1, 4, 26, 32]. The antioxidant compounds present in foods and beverages (polyphenols, vitamins, etc.) seem to contribute to plasma and possibly tissue total antioxidant capacity, reinforcing defenses against oxidative stress [10, 17, 22, 34] and preventing the mitochondrial chain components damage. In this direction, recently we have shown that alcohol-free beer intake reduces levels of oxidative damage in liver and heart mitochondria obtained from Adriamycin-treated rats [32].

V. Valls-Belles (✉) · C. Torres · P. Codoñer-Franch
Departamento de Pediatría, Ginecología y Obstetricia. Facultad de Medicina, Universidad de Valencia, Valencia, Spain
e-mail: victoria.valls@uv.es

P. Muñiz
Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, Burgos, Spain

On the other hand, the components of our diet are able to modify levels of mitochondrial membrane components. Therefore, the concentration of CoQ in rats is known to vary depending on nutritional status or environmental conditions. Changes in membrane CoQ concentration could be a result of changes in lipid environment such as, for example, peroxidation phenomena that take place at the membrane level [12].

Beer is a beverage with antioxidant capacity due its high content of vitamins, phenolic compounds, and melanoidins [10, 23]. This antioxidant capacity has been highly studied, showing the relation between beer intake and prevention of many human diseases mediated by free radicals [17, 32, 34].

We decided to use the anticancer agent Adriamycin as a model to study mitochondrial damage in rats because it induces cumulative dose-dependent cardiomyophathy, which has been ascribed to the mitochondrial redox-cycling of the drug. NADH dehydrogenase (complex I) of the mitochondrial electron transport chain and other oxidases are able of turning ADR into a semiquinone radical via univalent reduction [2]. This semiquinone radical can rapidly auto-oxidize using molecular oxygen as an electron acceptor, allowing for the initiation of a futile redox cycle resulting in a substantial increase in reactive oxygen species that affects proteins, lipids, etc., in close proximity to the site of generation. This mitochondrial dysfunction, including inhibition of components of mitochondrial respiration, has been implicated as a major determinant of pathogenesis of Adriamycin-induced pathologies [2, 31]. Furthermore, it is known that antioxidant compounds present in foods and beverages have a protective effect against damage to mitochondrial biomolecules [8, 19, 20, 25, 33].

In the light of these results, the aim of this study was to evaluate the effect of beer consumption on mitochondrial chain components in adriamycin-treated rats. Our hypothesis was that in Adriamycin-treated rats the in vivo consumption of beer results not only in a decreased oxidative stress, as we observed in a previous work [32], but also in the restoration of the activity and levels of mitochondrial membrane components. To prove our hypothesis we evaluated the effect of beer consumption on several parameters of respiratory chain function (complex I, complex IV) and on coenzymes Q_9 and Q_{10}.

Materials and methods

Chemicals and reagents

The following reagents were used in the experiments: Adriamycin (Pharmacia-Upjohn, Milan, Italy). Solvents and other reagents were obtained from Scharlau (Barcelona) and Merck (Darmstadt, Germany). All reagents were prepared with water purified by the Milli-Q system (Millipore Corp., Bedford, MA). NADH (Boehringer), Dodecyl-β-D-maltoside, cytocrome c; Coenzyme Q (Sigma).

Beer samples

Two types of lager beer were used in this study. Alcohol-free beer (alcohol levels below 0.5%) and alcohol beer (clear beer with alcohol content of 5.4%), both obtained from commercial sources in Spain. Beers were refrigerated and used immediately upon opening to prevent phenolic compounds oxidation and loss.

Experimental design

Wistar male rats of 3 months of age weighing between 250 and 300 g were housed in individual cages; maintained under controlled conditions of light cycle (12 h/12h light–dark) and temperature (22 °C); and fed a standard diet (IPM-20; Panlab Barcelona, Spain).

Rats were divided into six groups of eight rats. Rats were administered Adriamycin by intraperitoneal (i.p.) injection of two doses of 5 mg/Kg. A volume of 1.5 ml of beer was administered intragastrically using a probe. This volume is equivalent to a moderate intake (400 ml/day) in humans weighing 70 Kg.

Rats of group 1 (control group, C) were fed water and received two doses of isotonic saline solution (injected i.p. on days 7 and 14 of the study) for 21 days. Rats of group 2 (ADR group) drank water and were injected two doses of Adriamycin on days 7 and 14 of the study. Rats of groups 3 and 4 (Control + Beer groups) were fed alcohol-free beer or alcohol beer during 21 days and received two i.p. doses of saline solution. Rats of groups 5 and 6 (ADR + Beer) were injected with two doses of Adriamycin on days 7 and 14 of the study, and fed alcohol-free or alcohol beer for 21 days.

Preparation of mitochondria from rat heart and liver

Liver and heart mitochondria were isolated using the method described by Santos et al. [24]. Liver and heart tissues were excised, minced in ice-cold medium containing 250 mM sucrose, 1 mM EGTA, and 5 mM Hepes–KOH (pH 7.4), homogenized in a Potter-Elvehjen homogenizer, and then protease was added. Homogenates were centrifuged at 9,000 g for 10 min, low-speed supernatant was discarded, and the pellet was resuspended in isolation medium and centrifuged at 9,000 g for 10 min. The final mitochondrial pellets were resuspended in isolation medium.

Activity assays for mitochondrial respiratory complexes

Complex I: NADH-ubiquinone oxidoreductase activity (E.C. 1.6.5.3)

Complex I specific activity was determined by following oxidation of NADH at 340 nm (ε 6.81 $mM^{-1}cm^{-1}$) as described by Santos et al. [24]. 25 μL of heart mitochondria resuspension (1 mg/mL) was added to the reaction solution containing 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 60 mM coenzyme Q_{10}, and 2 μg/mL antimycin A, and was incubated at 25 °C for 1 min. Next, 10 μL of 75 μM NADH was added to start the reaction and initial rates of NADH oxidation were recorded during 1 min.

Complex IV: cytochrome oxidase (E.C. 1.9.3.1)

Complex IV activity was measured by following oxidation of cytochrome *c* (II) at 550 nm (ε 20 mM^{-1} cm^{-1}) [24]. The assay solution, which contained 20 mM potassium phosphate buffer (pH 7.0), 0.45 mM dodecyl-β-D-maltoside, and 15 μM reduced cytochrome *c* (II), was incubated for 30 s at 30 °C to determine the basal level of cytochrome *c* (II) oxidation. 5 μL of mitochondrial sample (0.6 mg/mL) was then added to start the reaction. The increased rate of cytochrome *c* (II) oxidation caused by the addition of mitochondrial sample was used to calculate complex IV activity.

Coenzyme Q_9 (CoQ_9) and coenzyme Q_{10} (CoQ_{10})

The ubiquinone content of the mitochondria was measured by reverse-phase HPLC analysis as previously indicated [14, 29]. The mitochondrial protein concentration used in the assay was of 1 mg/mL dissolved in 250 mM sucrose, 1 mM EGTA, and 5 mM Hepes–KOH (pH 7.4). After adding 2 mL of ethanol, hexane 2/5 v/v the samples were sonicated 2 min and centrifuged at 3,000 rpm for 3 min. The hexane phase was evaporated with nitrogen or air, and the pellet resuspended in 200 μL of ethanol. Quantification of CoQ_9 and CoQ_{10} levels was performed by HPLC–UV (275 nm) using two columns (Spherisorb ODS 1.50 × 0.46 cm and Kromasil 2.5 × 0.46 cm). The mobile phase was prepared by dissolving 7.0 g $NaCLO_4.H_2O$ in ethanol:methanol:70% $HCLO_4$ (699:300:1), with a flow rate of 1 mL/min.

Protein measurement

Total protein concentration was determined by the method of Lowry [16] using bovine albumin as a standard.

Statistical analysis

Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) for statistical evaluation of significant differences between two groups. The level of confidence required for significance was selected at $P < 0.05$. Statistical analysis was performed using the Statgraphics Plus 4.0 statistical package for windows (Statgraphics Plus for Windows 4.0. User's guide Manugistics, Inc., Rockville, MD, 1999).

Results

To evaluate the effect of intake of alcohol beer and alcohol-free beer on the mitochondrial respiratory chain components, we used the drug Adriamycin. The study was carried out in heart and liver mitochondria of different groups of rats: control group (C), controls fed alcohol or alcohol-free beer (C + Beer), rats treated with Adriamycin (ADR), and rats that treated with Adriamycin and fed alcohol or alcohol-free beer (ADR + Beer).

The results of the effect beer with and without alcohol intake on the mitochondrial respiratory chain are shown in Table 1. The parameters studied do not show any significant differences as result of beer intake during the period studied.

Table 1 Activity of complex I and complex IV and levels of coenzymes Q_9 and Q_{10} in liver and heart mitochondria of rats consuming alcoholic or alcohol-free beer

	Complex I (nmol/min × mg prot)		Complex IV (nmol/min × mg prot)		CoQ_9 (nmol/mg prot)		CoQ_{10} (nmol/mg prot)	
	Liver	Heart	Liver	Heart	Liver	Heart	Liver	Heart
C	283 ± 30	349 ± 46	1915 ± 298	2768 ± 267	8.23 ± 1.4	7.8 ± 0.5	0.60 ± 0.03	0.60 ± 0.06
C+ alcohol beer	254 ± 17	362 ± 31	1738 ± 292	2815 ± 150	7.7 ± 1.14	8.0 ± 1.5	0.80 ± 0.10	0.74 ± 0.04
C+ alcohol-free beer	259 ± 10	379 ± 29	1987 ± 66	2946 ± 162	8.9 ± 0.6	8.6 ± 1.4	0.73 ± 0.09	0.67 ± 0.18

Results are expressed as mean ± standard deviation. Control (C), and animals that consumed alcoholic beer (C+ Alcoholic beer) or alcohol-free beer (C+ alcohol-free beer)

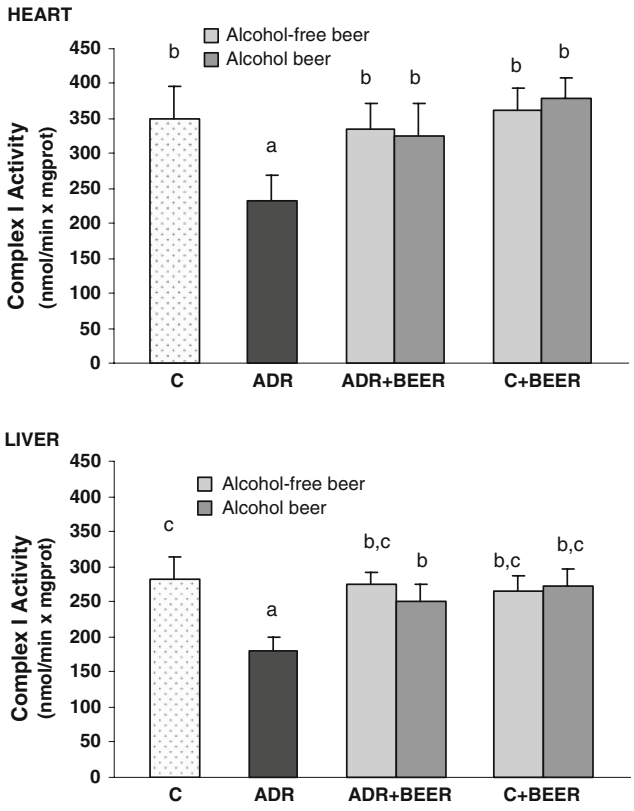

Fig. 1 Complex I activity in heart and liver mitochondria isolated from control (C), animals fed with beer (C + Beer), adriamycin-injected animals (ADR), and adriamycin-injected animals fed with beer (ADR + BEER). Values are expressed as mean ± standard deviation. The letters indicate significant differences ($P < 0.05$)

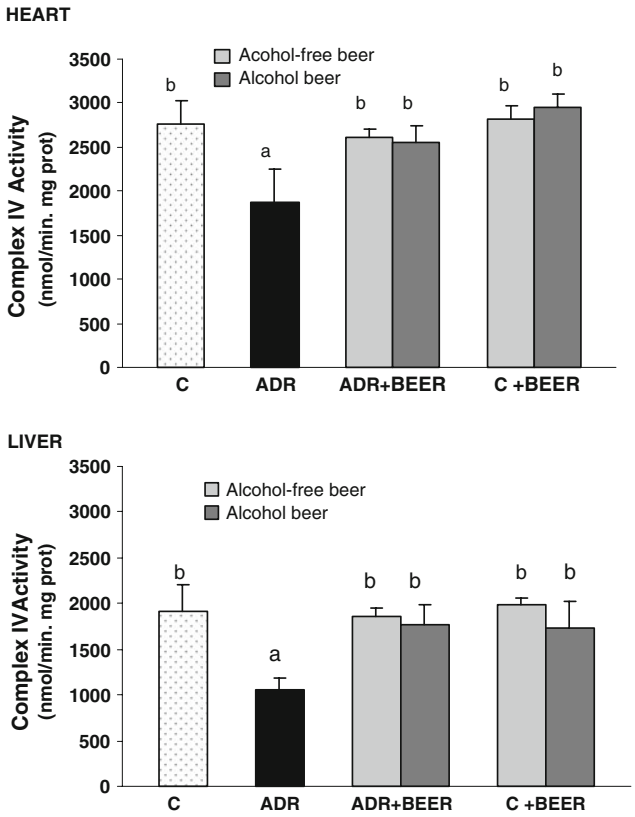

Fig. 2 Complex IV activity in heart and liver mitochondria isolated from control (C), animals fed with beer (C + Beer), adriamycin-injected animals (ADR), and adriamycin-injected animals fed with beer (ADR + BEER). Values are expressed as mean ± standard deviation. The letters indicate significant differences ($P < 0.05$)

Figure 1 shows the results of liver and heart mitochondrial complex I. Beer intake (C + Beer) did not induce significant changes in the activity of complex I. In the group of rats treated with Adriamycin, the activity of complex I was significantly decreased ($P < 0.005$) compared to the control group. This activity was around 33% lower in heart mitochondria and 45% lower in liver mitochondria compared to the control group. The intake of both alcohol and alcohol-free beer prevented the decrease in activity of heart mitochondrial enzymes with recovery values that reached 96 and 93% of those obtained for control group. Similar results were obtained in liver mitochondria where the intake of both alcohol-free and alcohol beer showed protective effects (of up to 91 and 97%, respectively).

The effects of Adriamycin treatment on complex IV of rat heart and liver mitochondria are shown in Fig. 2. No changes were observed in the C + Beer groups when compared to the control group (C). Adriamycin-treated rats decreased significantly ($P < 0.005$) their mitochondrial enzyme activity, showing decreases of around 32% in heart and around 45% in liver. Alcohol beer during the period studied prevented this Adriamycin-induced decrease (92%) in heart and liver mitochondria. Alcohol-free beer also showed this preventive effect (94 and 98% in heart and liver mitochondria, respectively).

Coenzyme Q is a component of the mitochondrial respiratory chain. In the present work, we have studied CoQ_{10} and CoQ_9, and the results are shown in Figs. 3 and 4, respectively. The intake of either alcohol or alcohol-free beer did not lead to changes in levels of the heart and liver coenzymes studied as shown by the results obtained when control group and beer intake groups are compared (C + Beer).

Levels of CoQ_{10} decreased significantly ($P < 0.005$) in rat treated with Adriamycin (Fig. 3). The percentage decrease was of 55% in heart mitochondria and of 41% in liver mitochondria. In the group of rats fed beer with or without alcohol (ADR + Beer) levels of CoQ_{10} were similar to those for the control group, showing higher increases in heart than in liver mitochondria.

The levels of CoQ_9 also decreased significantly in Adriamycin-treated rats. This decrease was higher in the heart, with a 55% lower CoQ_9 values, compared to the

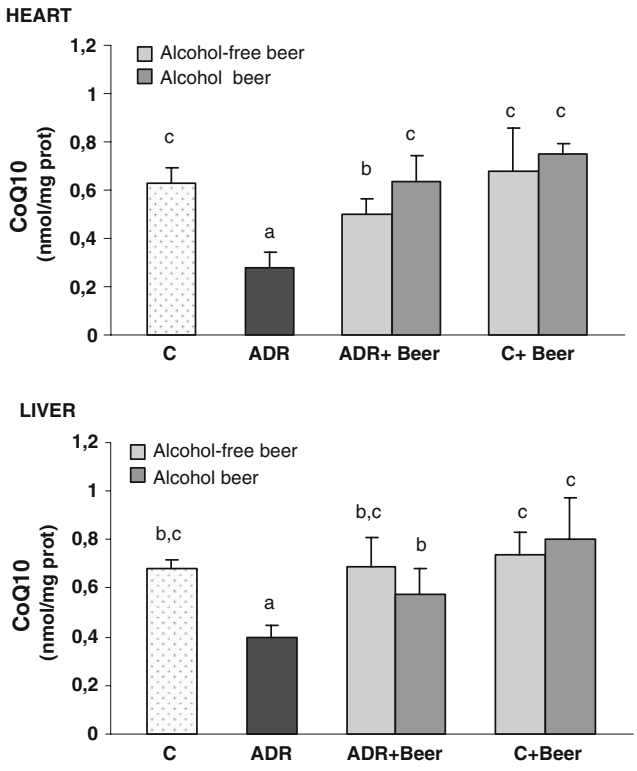

Fig. 3 Coenzyme Q_{10} activity in heart and liver mitochondria isolated from control (C), animals fed with beer (C + Beer), adriamycin-injected animals (ADR), and adriamycin-injected animals fed with beer (ADR + BEER). Values are expressed as mean ± standard deviation. The letters indicate significant differences ($P < 0.05$)

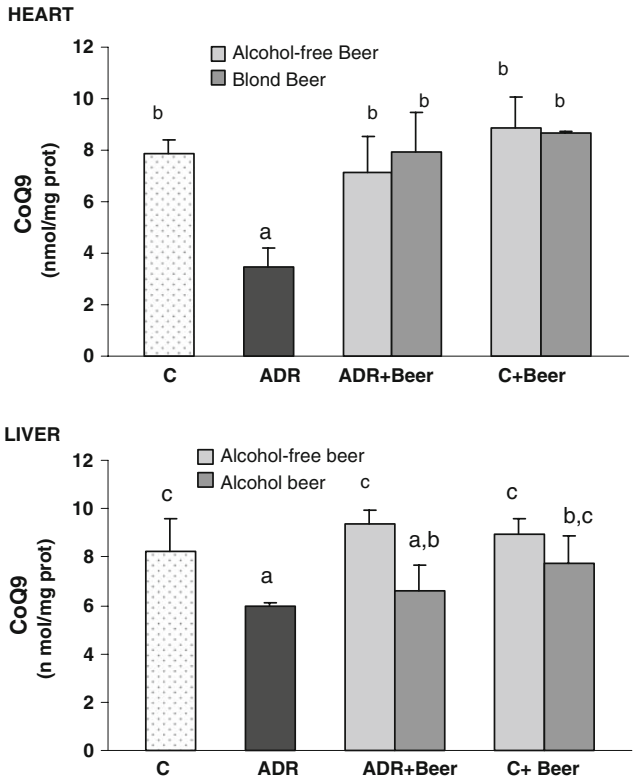

Fig. 4 Coenzyme Q_9 in heart and liver mitochondria isolated from control (C), adriamycin-injected animals (ADR), and adriamycin-injected animals fed with beer (ADR + BEER). Values are expressed as mean ± standard deviation. The letters indicate significant differences ($P < 0.05$)

liver, with a decrease of around 27%. Intake of both alcohol and alcohol-free beer prevented oxidation of CoQ_9 in rat heart mitochondria, with values that reached 100 and 90% of those obtained for control group, respectively. However, in the liver mitochondria alcohol-free beer showed a 100% prevention of CoQ_9 oxidation, however alcohol beer did not protect from oxidation, and no significant difference with the Adriamycin-treated group was observed.

Discussion

The present results indicate that consumption of beer, both with and without alcohol, decreased Adriamycin-induced damage of mitochondrial chain components.

In a previous study carried out by our research group [32], we observed that beer consumption reduces oxidative damage in mitochondria obtained from liver and heart of Adriamycin-treated rats. Interaction of Adriamycin with the mitochondrial membrane components is the main cause of free radicals generation, inducing changes in mitochondrial chain components. For this reason, in the present work we have studied if beer consumption also showed a protective effect on mitochondrial membrane components.

The hypothesis of the present research was that beer consumption could mitigate Adriamycin-mediated inhibition of mitochondrial respiratory function. Adriamycin generates oxygen free radicals by undergoing redox-cycling on complex I of the mitochondrial electron transport chain [7, 11, 35] giving as result the alteration of mitochondrial bioenergetics and of calcium regulation, which is a decisive factor in the pathogenic process [35]. Adriamycin-induced mitochondrial toxicity was confirmed by the changes in complexes I and IV activity induced by the drug, and a decrease in levels of CoQ_9 and CoQ_{10} in agreement with previous studies [2, 3, 31].

To evaluate the possible effect on energy levels, we studied the effect of consuming these beers on mitochondrial membrane components that play important roles in cell stability, such as complexes I and IV, and CoQ_9 and CoQ_{10}. Furthermore, the stability of these components is related to energy production (ATP) and to redox state of the cell. Beer is a beverage with high antioxidant capacity [23]. The effect of beer components depends on

bioavailability, membrane transport system, incorporation and stabilization of plasma membranes, and stability of the oxidation products [22].

Complex I catalyzes the futile redox-cycling of adriamycin to liberate oxygen free radicals, such as superoxide or hydroxyl radical [7]. During reverse electron transport Adriamycin inhibits NAD^+ reduction to NADH [6, 11] and alters the thiol-dependent regulation of different mitochondrial processes [27, 28]. Complex IV is a hemoprotein associated with the inner mitochondrial membrane. It is known that changes in Complex IV activity result in an alteration of the electron transport chain with an increase in the production of free radicals. Furthermore, changes or alterations in the mitochondrial membrane could be associated to an increase of the oxidative stress and lead to cell necrosis or apoptosis [18]. In this study, we observed that beer consumption prevented alterations in mitochondrial complexes I and IV, not finding any differences between alcohol and alcohol-free beer. These results could be indicating that beer components play a role not only in the protection of mitochondria against oxidative damage, but also in modulation of apoptosis or necrosis.

Coenzymes Q are components of the mitochondrial respiratory chain, distributed among the cellular membranes, that may provide protective benefits as antioxidants [5, 15, 21]. In eukaryotes, CoQ shuttles electrons from complexes I and II to complex III in the mitochondrial electron transfer system, and it has been shown that CoQ is an antioxidant which protects cells both directly, by preventing lipid peroxidation, and indirectly, by regenerating other antioxidants such as ascorbate and α-tocopherol [9, 20]. Furthermore, it is known that of CoQ_9 and CoQ_{10} concentration in rat liver changes depending on the tissues and on the nutritional and environmental conditions [30].

The results of our study show that levels of both coenzymes significantly decreased in heart and liver mitochondria as result of Adriamycin treatment. Beer intake had a protective effect avoiding the oxidation of both CoQ and, consequently, their decrease. This protective effect could be due to oxidative stress decrease in presence of antioxidants as result of beer intake or to the regeneration of coenzymes by effect of vitamin E [13]. In this sense, in a previous study we observed that levels of α-tocopherol are higher in rats fed with beer [3]. However, we observed no effects of alcohol beer in CoQ_9 levels of liver mitochondria, where no significant difference with the ADR group was observed, coinciding with the lower levels of α-tocopherol observed in this tissue [32]. This result could be to due to the central role played by liver in ethanol detoxification by means of different pathways, which results in an increase of reactive oxygen species that could provoke a decrease in levels of antioxidants such as CoQ_9 [9].

In conclusion, the results of the current investigation provide the first evidence of the restoration of mitochondrial respiration after Adriamycin-induced inhibition through beer consumption. Thus, beer may play a role in modulating the negative bioenergetic and functional consequences associated with Adriamycin treatment.

Acknowledgments The authors wish to express their gratitude to the information centre "Centro de Información Cerveza y Salud" for their financial support.

References

1. Addolorato G, Leggio L, Ojetti V, Capristo E, Gasbarrini G, Gasbarrini A (2008) Effects of short-term moderate alcohol administration on oxidative stress and nutritional status in healthy males. Appetite 50(1):50–56
2. Bagchi D, Bagchi M, Hassoun EA, Kelly J, Stohs SJ (1995) Adriamycin-induced hepatic and myocardial lipid peroxidation and DNA damage an enhanced excretion of urinary lipid metabolites in rats. Toxicology 95:1–9
3. Barogi S, Baracca A, Cavazoni M, Castelli G, Lenaz G (2000) Effect of the oxidative stress induced by adriamycin on rat hepatocytes bioenergetics during ageing. Mech Ageing Dev 113:1–21
4. Bruckdorfer KR (2008) Antioxidants and CVD. Proc Nutr Soc 67(2):214–222
5. Crane FL (2001) Biochemical functions of coenzyme Q_{10}. J Am Coll Nutr 20:591–598
6. Davies KJ, Doroshow JH (1986) Redox cycling of anthracyclines by cardiac mitochondira I. Antracycline radical formation by NADH dehydrogenase. J Biol Chem 261:3060–3067
7. Doroshow JH (1983) Antracycline antibiotic-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. Cancer Res 43:4543–4551
8. Du Y, Lou H (2008) Catechin and proanthocyanidin B4 from grape seeds prevent doxorubicin-induced toxicity in cardiomyocytes. Eur J Pharmacol 4; 591(1–3):96–101
9. Genova ML, Pich MM, Biondi A, Bernachia A, Falasca A, Bovina C, Formigini G, Castelli G, Lenaz G (2003) Mitochondrial production of oxygen radical species and the role of Coenzyme Q as an antioxidant. Exp Biol Med 228(5):506–513
10. Ghiselli A, Natella F, Guidi A, Montanari L, Fantozzi P, Scaccini C (2000) Beer increases plasma antioxidant capacity in humans. J Nutr Biochem 11:76–80
11. Guzy J, Kusnir J, Marekova M, Chavkova Z, Dubayova G, Mojzisova L, Mirossay J, Mojzis J (2003) Effect of quercetin on Daunorubicin-induced heart mitochondria change in rats. Physiol Res 52:573–780
12. Huertas JR, Battino M, Lenaz G, Mataix FJ (1991) Changes in mitochondrial and microsomal rat liver coenzyme Q_9 and Q_{10} conten induced by dietary fat and endogenous lipid peroxidation. FEBS 287:89–92
13. Kagan V, Serbinova E, Packer L (1990) Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. Biochem Biophys Res Común 169:851–857
14. Lass A, Sohal RS (1999) Comparisons of coenzyme Q bound to mitochondrial membrane proteins among different mammalian species. Free Rad Biol Med 27(1–2):220–226
15. Lenaz G, Fato R, Formiggini G, Genova ML (2007) The role of Coenzyme Q in mitochondrial electron transport. Mitochondrion 7:S8–S33

16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
17. Martinez-Alvarez JR, Valls-Belles V, López-Jaén M, Villariño-Marín A, Codoñer-Franch P (2009) Effects of alcohol-free beer on lipid profile and parameters of oxidative stress and inflammation in elderly women. Nutrition 25(2):182–187
18. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B (2007) Mitochondria, oxidative stress and cell death. Apoptosis 12(5):913–922
19. Psotova J, Clopeikova S, Miketova P, Simanek V (2004) Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Phyto Res 18:516–521
20. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC (2002) Antioxidant nutrients and adriamycin toxicity. Toxicology 30–180(1):79–95
21. Quiles JL, Ochoa JJ, Battino M, Gutierrez-Rios P, Nepomuceno EA, Frías ML, Huertas JR, Mataix J (2005) Life-long supplementation with a low dosage of coenzyme Q10 in the rat: effects on antioxidant status and DNA damage. Biofactors 25(1–4):73–86
22. Rice-Evans C (2001) Flavonoids antioxidants. Curr Med Chem 8:797–807
23. Rivero D, Perez-Magarino S, Gonzalez-Sanjosé ML, Valls-Belles V, Codoñer P, Muniz P (2005) Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and melanoidins. J Agric Food Chem 53(9):3637–3642
24. Santos DL, Moreno AJM, Leino RL, Frobeg MK, Guayanes KB (2002) Carvedilol protects against doxorubicin-induced mitochondrial cardiomyopathy. Toxicol Applied Pharmacol 185:218–227
25. Sharma G, Tyagi A, Singh RP, Chan DCF, Agarwal R (2004) Synergistic anticancer effects of grape seed extract and conventional cytotoxic agent doxorobucin against human breast carcinoma cells. Breast Cancer Res Treat 85:1–12
26. Shigenaga MK, Hage TM, Ames BN (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 91:10771–10778
27. Sokolove PM (1988) Mitochondrial sulfhydryl group modification by adriamycin aglycones 1988. FEBS Lett 234:199–202
28. Solem LE, Wallace KB (1993) Selective activation of the sodium independent, cyclosporin A-sensitive calcium pore of cardiac mitochondria by doxorubicin. Toxicol Appl Pharmacol l121:50–57
29. Takada M, Ikenoya S, Yuzuriha T, Katayama K (1984) Simultaneous determination of reduced and oxidized ubiquinones. Methods Enzymol 105:147–155
30. Tang PH, Miles MV, Miles L, Quinlan J, Wong B, Wenisch A, Bove K (2004) Measurement of reduced and oxidized coenzyme Q_9 and coenzyme Q_{10} levels in mouse tissues by HPLC with coulometric detection. Clin Chim Acta 341(1–2):173–184
31. Valls-Belles V, Castellucio C, Fato R, Genova ML, Bovina C, Sáez G, Marchetti M, Parenti-Castelli G, Lenaz G (1994) Protective effect of exogenous coenzyme Q against damage in rat liver. Biochem Mol Biol Inter 33(4):633–642
32. Valls-Belles V, Torres MC, Boix L, Muñiz P, González-SanJose ML, Codoñer-Franch P (2008) Tocopherol, MDA–HNE and 8-OHdG levels in liver and heart mitochondria of adriamycin-treated rats fed with alcohol-free beer. Toxicology 249:97–101
33. Valls-Belles V, Torres MC, Muñiz P, Beltran S, Martinez-Alvarez JR, Codoñer-Franch P (2006) Defatted milled graped seed protects adriamycin-treated hepatocytes. Eur J Nutr 45(5):251–258
34. Vinson JA, Mandarano M, Hirst M, Trevithick JR, Bose P (2003) Phenol antioxidant quantity and quality in foods: beers and the effect of two types of beer on animal model of atherosclerosis. J Agric Food Chem 51:5528–5533
35. Wallace RB (2008) Mitochondrial off targets of drug therapy. Pharmacol Toxicol 29(7):361–366