FEBS 28997 FEBS Letters 578 (2004) 53–57

Biological validation of coenzyme Q redox state by HPLC-EC measurement: relationship between coenzyme Q redox state and coenzyme Q content in rat tissues

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Received 9 July 2004; revised 16 October 2004; accepted 18 October 2004

Available online 4 November 2004

Edited by Richard Cogdell

Abstract The properties of coenzymes Q (CoQ9 and CoQ10) are closely linked to their redox state (CoQox/total CoQ)×100. In this work, CoQ redox state was biologically validated by high performance liquid chromatography-electrochemical measurement after modulation of mitochondrial electron flow of cultured cells by molecules increasing (rotenone, carbonyl cyanide chlorophenylhydrazone) or decreasing (antimycin) CoQ oxidation. The tissue specificity of CoQ redox state and content were investigated in control and hypoxic rats. In control rats, there was a strong negative linear regression between tissular CoQ redox state and CoQ content. Hypoxia increased CoQ9 redox state and decreased CoQ9 content in a negative linear relationship in the different tissues, except the heart and lung. This result demonstrates that, under conditions of mitochondrial impairment, CoQ redox control is tissue-specific.

Keywords: Coenzyme Q9; Coenzyme Q10; Redox state; High performance liquid chromatography-electrochemical; Hypoxia; Rat

1. Introduction

Coenzyme Qs (CoQs) are strongly implicated in the oxido-reduction cellular metabolism. The length of the constitutive isoprenoid side chain determines the homologous forms of CoQs. CoQ10 is the major form in human cells and plants, whereas CoQ9 predominates in rodents [1].

First, CoQs are the only non-protein components of the respiratory chain. They are bound to mitochondrial membrane proteins [2] and function as an obligatory electron carrier between complexes I or II and complex III of the mitochondrial respiratory chain. At this site, partial oxidation of CoQred produces a semi-reduced form, which interacts with oxygen to generate superoxide anion. This accounts for the major part of superoxide anion physiologically generated by the mitochondria. Second, in their CoQox, CoQs behave as an obligatory cofactor of uncoupling protein activity [3]. Last, CoQs are the only lipophilic antioxidants which are biosynthesized in humans. In their reduced form they have an antioxidant activity, as scavengers of reactive oxygen species or lipid radicals and regenerators of α-tocopherol from the α-tocopheroxyl radical [4]. Moreover, CoQ redox state may be an useful marker of oxidative stress [5,6].

Various studies have reported beneficial effects of CoQ10 supplementation in different disease conditions during animal experimentation [7,8] or human therapy [9,10]. As described above, these lipophilic and ubiquitous molecules have functions related to their redox state. To investigate CoQ content and redox state, a sensitive and reliable method of quantifying oxidized and reduced forms is required.

Several high performance liquid chromatography (HPLC) methods have been described for the determination of total CoQ10 [11–16]. CoQ10ox and CoQ10red have also been measured using complex analytical procedures [12,16,17]. At the present time, EC detection is the most sensitive of the different modes of detection coupled with HPLC. Recently, Tang et al. [6,18] developed a simple and rapid HPLC-electrochemical (EC) method for the determination of CoQsox and CoQsred.

The aim of this work was to (i) biologically validate HPLC-EC measurement of CoQ redox state by using molecules which can modify mitochondrial CoQ oxidation and (ii) investigate the tissular specificity of CoQ redox state and content in control and hypoxic rats. Hypoxia is in fact well known to be responsible for mitochondrial oxidative stress [19], which could modify CoQ redox state.

2. Materials and methods

2.1. Materials

CoQ9ox, CoQ10ox and other chemicals were obtained from Sigma (L'Isle d'Abeau Chesnes, France). All chemicals were of HPLC grade.

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Abbreviations: CoQs, coenzymes Q; CoQ9, coenzyme Q9; CoQ10, coenzyme Q10; CoQred, reduced CoQ; CoQox, oxidized CoQ; HPLC, high performance liquid chromatography; EC, electrochemical; CCCP, carbonyl cyanide chlorophenylhydrazone; PBS, phosphate buffer saline

0014-5793/$22.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2004.10.067

2.2. Cell culture conditions for biological validation of CoQ redox state

Murine 3T3-F442A preadipocytes were routinely cultured as previously described [20]. Cells were seeded at a density of 4500 cells/cm^2 in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Measurements were performed at a pre-confluent state. Molecules acting at different levels of the mitochondrial respiratory chain were chosen to increase or decrease CoQ oxidation. Rotenone, an inhibitor of complex I, and carbonyl cyanide chlorophenylhydrazone (CCCP), an uncoupler, increase CoQ oxidation. On the contrary, antimycin, an inhibitor of complex III, decreases CoQ oxidation. Molecules were dissolved in ethanol to a final concentration of 0.4% in the medium. Controls were performed with 0.4% ethanol. These molecules were used at concentrations which only moderately affect cell respiration: rotenone (10^{-9} M) and antimycin (2×10^{-8} M) which inhibit cell respiration by 15%, and CCCP (10^{-6} M) which increases it by 15% were added to the medium. At different times, cells were washed twice with phosphate buffer saline (PBS), trypsinized and collected in PBS. After centrifugation ($400 \times g$,10 min), the pellet was homogenized in 100 μl of PBS and stored at −80 °C. This freezing procedure was validated by comparison with measurement of freshly prepared homogenates.

2.3. Animals and treatments

Male Wistar rats (220–260 g) were fed ad libitum. In a first experiment, 6 rats were killed by exsanguination. The dissected organs were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. In a second experiment, three rats were exposed to hypoxia in a hypobaric chamber simulating 8000 m above sea level (270 mmHg, 21% O_2) for 4 h (acute hypoxia) and three control rats breathed ambient air. After exsanguination, dissected organs were treated as described above.

2.4. Quantification of CoQs (reduced and oxidized forms)

Extraction of biological samples. In order to define the best extraction conditions for preventing CoQ oxidation, two comparative assays were performed with 3T3-F442A cells (minimum 250 000 cells). In the first assay, 100 μl of cell homogenate was mixed with 500 μl of ethanol and centrifuged ($4000 \times g$, 3 min). The supernatant was extracted with 2 ml of hexane. After evaporation of the hexane phase, the pellet was dissolved in 500 μl of the chromatographic mobile phase (see HPLC-EC apparatus below). In the second assay, 100 μl of cell homogenate was mixed with 500 μl of 2-propanol, centrifuged ($4000 \times g$, 3 min) and the supernatant was recovered. 50 μl of mobile phase (first assay) or of the 2-propanol extract (second assay) was injected into the HPLC system.

In addition, to evaluate CoQ content recovery and CoQred conservation during 2-propanol extraction, measurements were done with cellular homogenates with or without a known quantity of CoQred standard (107 nmol).

For the subsequent experiments, 2-propanol extraction was used. Cell homogenates were extracted as described above. Frozen tissues (100 mg) were added to 0.9 ml of 2-propanol and homogenized with an Ultraturax blender. 100 μl of this homogenate was mixed with 500 μl of 2-propanol and treated as described above.

HPLC-EC apparatus. This consisted of a Gilson 307 pump, a Rheodine injector, an analytical column and an ESA Coulochem 2 Electrochemical Detector (Model 5200 A) with a Pentium II computer/controller with EuroChrom 2000 Integration Package for Windows. Briefly, a guard cell (ESA Model 5020) (E1) was placed between the pump and the injector to oxidize electroactive materials in the mobile phase. The analytical cell (ESA Model 5010-porous graphite) consisted of a series of two coulometric electrodes and was connected in series to the analytical column: the first electrode (E2) was for CoQ reduction and the second electrode (E3) was for CoQred detection. The different homologs, CoQ9 and CoQ10, were identified by chromatographic separation. In-prefilters were placed between the pump and the guard cell and between the analytical column and the analytical cell. The analytical column was a reverse-phase Hypersil BDS C18 column (4 mm × 25 cm, 5 μm beads). The mobile phase for isocratic elution of CoQ9 and CoQ10 contained sodium acetate (3.4 g), 8 mL of acetic acid, 8 mL of 2-propanol, 344 mL of methanol and 80 mL of hexane. The flow rate of the mobile phase (degassed before use) was 1 mL/min. The HPLC-EC system was set at room temperature (21 ± 1 °C).

Preparation of calibrators. Oxidized and reduced forms of CoQs were identified and quantified using self-made external calibrators. In an amber balloon, 2 mg of CoQox (Q9 or Q10) was dissolved in 100 mL of 2-propanol. Concentrations of these stock solutions were confirmed by measuring absorbance at 275 nm and by reference to known extinction coefficients ($E^{1\%}_{1cm}$185 for CoQ9 and 165 for CoQ10). Five ml of the CoQox stock solutions was diluted in the mobile phase (v/v), aliquoted and frozen at −80 °C. The rest (95 ml) of each stock solution was reduced in a loop to −1000 mV in the system pump-guard cell maintained in the dark for approximately 3 h. The conversion rate of CoQox to CoQred was around 99%. After 6 months at −80 °C, a loss of only 2% of CoQred was observed in the reduced stock solutions.

Coulometric detection. To optimize the applied EC potentials in our HPLC conditions, a hydrodynamic voltamogram was obtained by repeated injections for all compounds studied. On the basis of the assessed hydrodynamic voltamogram, and to oxidize any electrochemically active eluate, the guard cell potential was always set at +1000 mV. The E2 and E3 cell potentials were set at −650 and +420 mV, respectively. EC detection in the analytical cell was carried out after specific reduction of CoQox by E2. Oxidation by E3 then enabled electron flow to be detected.

2.5. CoQ redox state

This was calculated for CoQ9 and CoQ10 as: ([CoQox]/[total CoQ]) × 100 with [CoQox] + [CoQred] = [total CoQ].

2.6. Statistical analysis

Means ± S.E.M. were calculated and statistically significant differences between two groups were determined by Student's t test at $P < 0.05$.

2.7. External analytical quality control

Since 2003, the results of this technique are compared with the analytical quality program organized by the National Institute of Standard Technology (NIST) (Gaithersburg, USA) on lyophilized serums. The results are in complete agreement with the median of a four laboratories interassay.

3. Results and discussion

3.1. Analytical conditions

Calibration, reproducibility and sensitivity of chromatographic analysis. CoQ calibrator solution (mixture of the four diluted stock solutions) was prepared in 2-propanol (or in

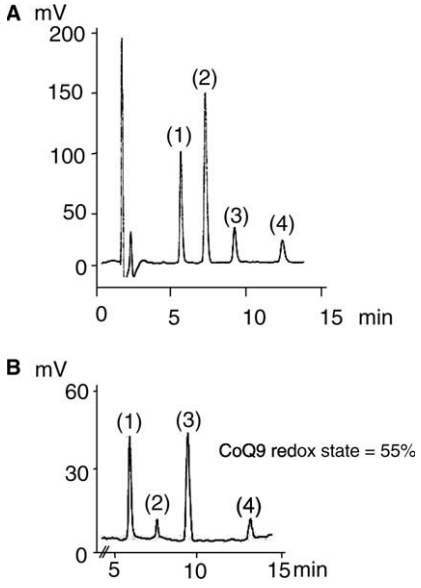

Fig. 1. Typical HPLC-EC chromatogram of CoQ9 and CoQ10. (A) Calibrators at the following concentrations: 1545 nM for CoQ9red (1), 1576 nM for CoQ10red (2), 1180 nM for CoQ9ox (3) and 1113 nM for CoQ10ox (4). (B) Cell homogenate after 2-propanol extraction.

ethanol/hexane) as for biological samples. Fig. 1A shows a typical chromatogram. The two forms of each CoQ were well separated from one another. Analysis was fast, with retention times of 6.0, 7.5, 9.0, and 13.5 min, respectively, for CoQ9red, CoQ10red, CoQ9ox, and CoQ10ox. For the 4 forms, calibration curves were performed with three different calibrator solutions. An excellent linear relationship ($r^2 = +0.99$) was observed between the area of the peak (mV/min) and the molar concentration ratio of each compound over a wide concentration range (from 10 to 3000 nM). Variation coefficients were 2.9, 3.5, 3.5 and 3.7% for CoQ9red, CoQ10red, CoQ9ox and CoQ10ox, respectively. The limits of detection were 21 pmol for CoQsred and 15 pmol for CoQsox.

Extraction of biological samples. The two extraction conditions were used for quantification of either total CoQ9 or CoQ10 [2,6,21,22]. However, it was observed that ethanol/hexane extraction converted the CoQs to the oxidized form [23].

Fig. 1B shows a typical chromatogram obtained with 2-propanol extraction. Total CoQ9 (ox and red) recovery was 41.9 ± 4.2 pmol/10^6 cells and was not significantly different from that obtained with ethanol/hexane extraction (38.6 ± 3.5) (results not shown). However, there was more CoQ9red (and less CoQ9ox) with 2-propanol extract than with ethanol/hexane extract (results not shown). In this way, the values of redox state were 78% with ethanol/hexane and 55% with 2-propanol. These results show that, compared with ethanol/hexane extraction, 2-propanol extraction protects CoQ from oxidation.

Moreover, when 107 nmol of CoQ9red standard were added to a cell homogenate and extracted with 2-propanol, total CoQ9 and CoQ9red recoveries were $98 \pm 2\%$ and $96 \pm 1\%$, respectively (results not shown).

Thus, 2-propanol extraction reliably measured CoQ content or CoQ redox state for biological samples and was used for the subsequent experiments.

3.2. Biological validation of CoQ redox state by modulation of mitochondrial electron flow

The measurements of CoQ redox state were biologically validated using various molecules well known to modify electron flow at different levels of the respiratory chain. Thus,

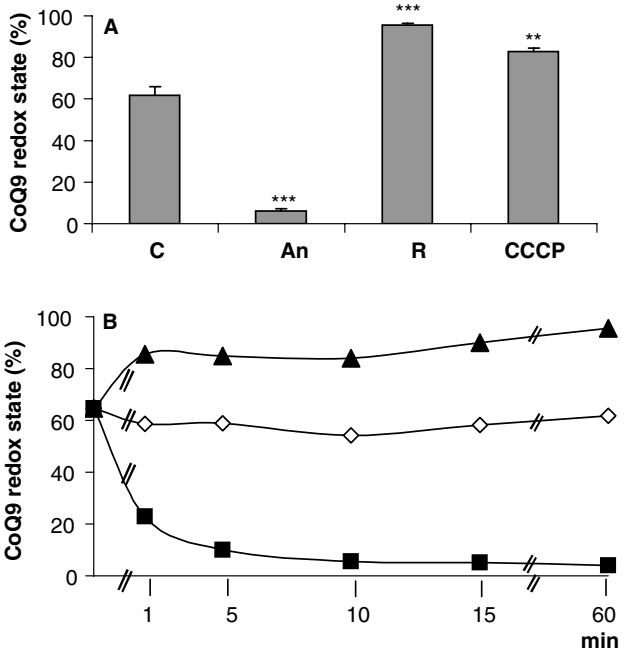

Fig. 2. CoQ9 redox state after modulation of the mitochondrial respiratory chain electron flow of cultured cells. (A) Cells were treated 60 min with antimycin (An, 2×10^{-8} M), rotenone (R, 10^{-9} M), CCCP (10^{-6} M) or 0.4% ethanol (control, C). (B) Cells were treated between 1 and 60 min with An 2×10^{-8} M (■), R 10^{-9}M (▲) or 0.4% ethanol (control, ◇). These concentrations of An and R inhibit cell respiration by 15% and the concentration of CCCP increases it by 15%. Results are expressed as (A) means ± S.E.M. (four independent experiments) or (B) means (two independent experiments). $^{**}P < 0.01$ and $^{***}P < 0.001$ versus (C).

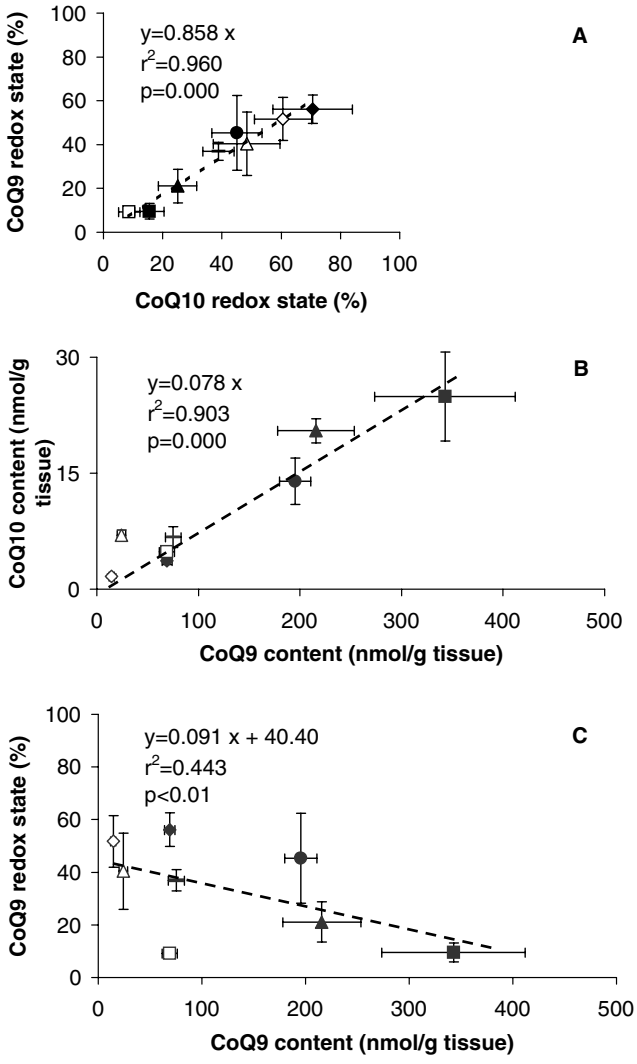

Fig. 3. Linear regression between CoQ redox state, CoQ content or between CoQ9 content and CoQ9 redox state in tissues* of control rats. (A) CoQ9 redox state as a function of CoQ10 redox state, (B) CoQ10 content as a function of CoQ9 content and (C) CoQ9 redox state as a function of CoQ9 content in lung (◇), spleen (△), glycolytic muscle (◆), oxidative muscle (–), heart (●), kidney (▲) and liver (■). *Testis (□) was not taken into consideration for the determination of linear regression between CoQ9 redox state and CoQ9 content. Results are expressed as means ± S.E.M. for six rats.

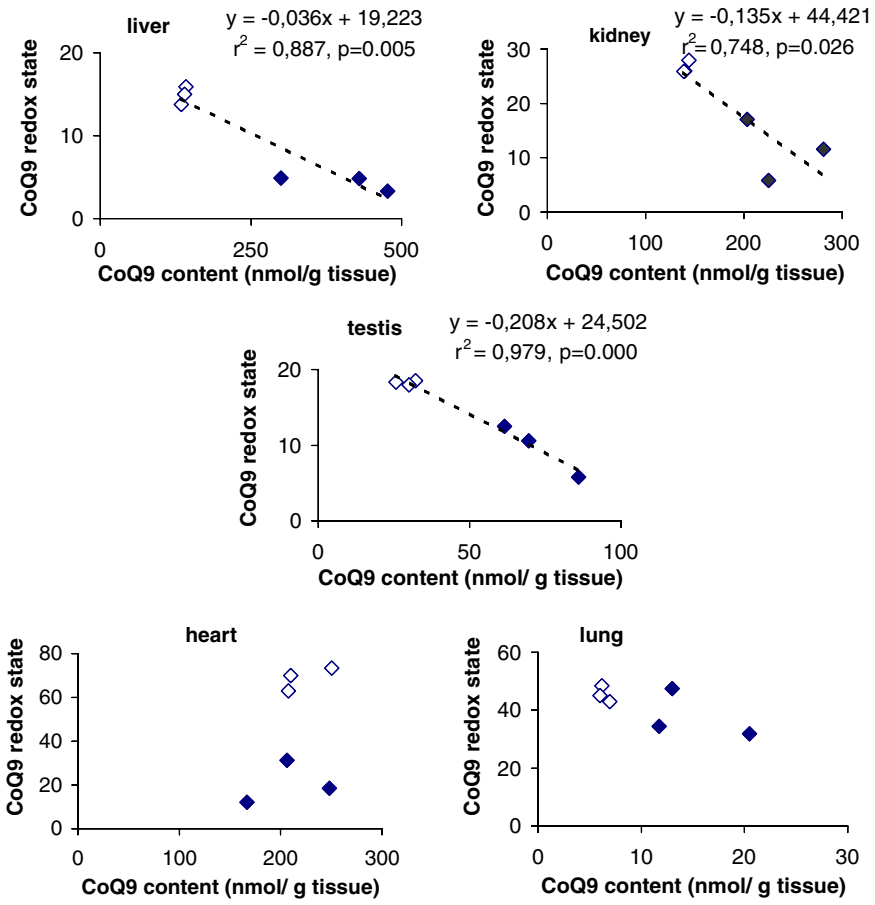

Fig. 4. Relationship between CoQ9 redox state and CoQ9 content in tissues of control rats (◆) and rats subjected to hypoxia (◇) ($n = 3$ in each group).

rotenone and antimycin, inhibitors of complexes I and III, respectively, were chosen to inhibit mitochondrial electron flow and CCCP to increase it [24–26]. By inhibiting electron flow at the level of complex I, rotenone limits CoQ reduction and enhances CoQ oxidation. On the other hand, antimycin, by blocking the electron flow at the level of the Q-cycle, helps to maintain CoQ reduction. CCCP, acting as a protonophore, dissipates the transmembrane proton gradient, reduces the constraints on the mitochondrial respiratory chain and enhances CoQ oxidation. Fig. 2A illustrates the effects of these molecules on CoQ9 redox state after one hour of incubation. As expected, CoQ9 redox state was significantly decreased in the presence of antimycin and significantly increased in the presence of rotenone and CCCP. Similar results were obtained with CoQ10 (results not shown). The kinetics of the CoQ redox states were determined between 1 and 60 min after addition of rotenone or antimycin. These molecules induced a marked modification of CoQ9 redox state within 1 min (Fig. 2B). Similar results were obtained with CoQ10 (results not shown).

These results represent detection of the reduced and oxidized forms as expected under the experimental conditions described. They biologically validate the measurement of CoQs redox states in complex biological matrixes despite sample handling. It was noteworthy that a slight change in respiratory chain activity rapidly influenced the whole cellular CoQ redox state.

3.3. Relationship between CoQ redox state and CoQ content in rat control tissues

Fig. 3A shows a positive linear regression between CoQ9 redox state and CoQ10 redox state of different tissues (slope = 0.858). CoQ9 and CoQ10 redox states thus have approximatively the same value for a given tissue, but values vary between different tissues. On the whole, we obtained higher proportions of reduced CoQ than authors who used a more complex extraction method and HPLC-UV detection [27]. However, we observed proportions of oxidized CoQ9 similar to those obtained recently in mouse tissues (lung, liver and muscle) [18]. Fig. 3B shows a positive linear regression between CoQ10 content and CoQ9 content. So, in each tissue, CoQ9 and CoQ10 contents vary in the same proportion. We observed that each tissue is characterized not only by its redox state but also by its CoQ9 content. For example, liver and lung (Fig. 3C) have the highest and lowest CoQ9 contents but are characterized by a low and a high redox state, respectively. Testis CoQ9 seems protected from oxidation, perhaps by the presence of high levels of other antioxidants, such as α-tocopherol as described in spermatozoa [28]. Finally, we demonstrated for the different tissues (testis excluded) a negative linear regression between CoQ9 redox state and CoQ9 content (Fig. 3C) and between CoQ10 redox state and CoQ10 content (results not shown).

These results indicate that for most rat control tissues, CoQ redox state is strongly linked to CoQ content, for both CoQ9

and CoQ10. It appears that, in physiological conditions, tissular CoQ content determines the CoQ redox state (or reciprocally).

3.4. CoQ redox state and CoQ content in tissues of rats subjected to hypoxia

Hypoxia occurs in various conditions (vascular and pulmonary diseases or cancer) [29] and is associated with mitochondrial oxidative stress [19] whose intensity varies according to the tissue. After hypoxia, CoQ10 was modified only in the heart. This tissue showed significantly higher values of CoQ10 content and CoQ10 redox state than controls (results not shown).

Fig. 4 shows the relationship between CoQ9 redox state and CoQ9 content in different tissues (liver, kidney, testis, heart and lung) of rats under hypoxic or normoxic conditions. Comparatively to control tissues, liver, kidney and testis of rats under hypoxia showed higher values in CoQ9 redox state ($4.34 \pm 0.52\%$ versus 14.90 ± 0.62, $P < 0.001$; 11.48 ± 3.25 versus 26.59 ± 0.67, $P < 0.05$; 9.62 ± 2.00 versus 18.45 ± 0.10, $P < 0.05$ respectively) and lower values in CoQ9 content (402.08 ± 52.84 nmol/g of tissue versus 138.97 ± 2.24, $P < 0.05$; 236.38 ± 23.06 versus 140.85 ± 1.56, $P < 0.05$; 72.37 ± 7.23 versus 28.90 ± 3.23, $P < 0.05$). Moreover, there was a negative linear regression between these two parameters. This increase in CoQ9 redox state is consistent with the mitochondrial oxidative stress described during hypoxia [19]. Comparatively to controls, heart from rats under hypoxia showed high values in CoQ9 redox state ($20.69 \pm 5.60\%$ versus 68.83 ± 3.08, $P < 0.01$) and similar values in CoQ9 content (207.03 ± 23.56 nmol/g of tissue versus 222.77 ± 13.83, ns). Comparatively to controls, lung from hypoxic rats showed similar values in CoQ9 redox state ($37.92 \pm 4.83\%$ versus 45.47 ± 1.63, ns) and lower values in CoQ9 content (15.07 ± 2.74 nmol/g of tissue versus 6.38 ± 0.29, $P < 0.05$). However, no significant linear regression between CoQ9 redox state and CoQ9 content was observed in heart and lung. This can be explained by different adaptive responses of tissues for these CoQ parameters during low oxygen tension. Indeed, it is well known that some tissues (lung, heart) are able to extract variable oxygen concentrations and then adapt well to hypoxia [30]. Our results agree with a previous study in which HIF-1α, a marker reciprocally regulated by oxygen tension, was strongly detected in liver, kidney, spleen and muscle of mice subjected to 6% hypoxia but was present at a low level in the heart and absent in the lung [31].

In conclusion, this study suggests that evaluation of CoQ content and redox state is necessary to improve our knowledge of cellular CoQ regulation. Moreover, it emphasizes the tissue specificity of CoQ redox control in pathological situations associated with mitochondrial dysfunction.

Acknowledgements: This research was supported in part by the Agence Nationale de Recherche sur le Sida (2001/198). We thank M.C. Prevost for help in experimental hypoxia and M.C. Carmona for critical comments. We are also grateful to N. Crowte for help in translation of the manuscript.

References

[1] Battino, M., Ferri, E., Gorini, A., Federico Villa, R., Rodriguez Huertas, J.F., Fiorella, P., Genova, M.L., Lenaz, G. and Marchetti, M. (1990) Membr. Biochem. 9, 179–190.
[2] Lass, A. and Sohal, R.S. (1999) Free Radic. Biol. Med. 27, 220–226.
[3] Echtay, K.S., Winkler, E., Frischmuth, K. and Klingenberg, M. (2001) Proc. NAtl. Acad. Sci. USA 98, 1416–1421.
[4] Genova, M.L., Pich, M.M., Biondi, A., Bernacchia, A., Falasca, A., Bovina, C., Formiggini, G., Parenti Castelli, G. and Lenaz, G. (2003) Exp. Biol. Med. 228, 506–513.
[5] Yamamoto, Y. and Yamashita, S. (1997) Mol. Aspects Med. 18, s79–s84.
[6] Tang, P.H., Miles, M.V., DeGrauw, A., Hershey, A. and Pesce, A. (2001) Clin. Chem. 47, 256–265.
[7] Rauscher, F.M., Sanders, R.A. and Watkins, J.B. III (2001) J. Biochem. Mol. Toxicol. 15, 41–46.
[8] Kwong, L.K., Kamzalov, S., Rebrin, I., Bayne, A.C., Jana, C.K., Morris, P., Forster, M.J. and Sohal, R.S. (2002) Free Radic. Biol. Med. 33, 627–638.
[9] Geromel, V., Darin, N., Chrétien, D., Bénit, P., DeLonlay, P., Rötig, A., Munnich, A. and Rustin, P. (2002) Mol. Genet. Metab. 77, 21–30.
[10] Hodgson, J.M., Watts, G.F., Playford, D.A., Burke, V. and Croft, K.D. (2002) Eur. J. Clin. Nutr. 56, 1137–1142.
[11] Motchnik, P.A., Frei, B. and Ames, B.N. (1994) Methods Enzymol. 234, 269–279.
[12] Podda, M., Weber, C., Traber, M.G. and Packer, L. (1996) J. Lipid Res. 37, 893–901.
[13] Wakabayashi, H., Yamato, S., Nakajima, M. and Shimada, K. (1994) Biol. Pharm. Bull. 17, 997–1002.
[14] Lagendijk, J., Ubbink, J.B. and Vermaak, W.J. (1996) J. Lipid Res. 37, 67–75.
[15] Finckh, B., Kontush, A., Commentz, J., Hubner, C., Burdelski, M. and Kohlschutter, A. (1995) Anal. Biochem. 232, 210–216.
[16] Lang, J.K. and Packer, L. (1987) J. Chromatogr. 385, 109–117.
[17] Wang, Q., Lee, B.L. and Ong, C.N. (1999) J. Chromatogr. B 726, 297–302.
[18] Tang, P.H., Miles, M.V., Miles, L., Quinlan, J., Wong, B., Wenisch, A. and Bove, K. (2004) Clin. Chim. Acta 341, 173–184.
[19] Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M. and Schumacker, P.T. (2000) J. Biol. Chem. 275, 25130–25138.
[20] Carriere, A., Fernandez, Y., Rigoulet, M., Penicaud, L. and Casteilla, L. (2003) FEBS Lett. 550, 163–167.
[21] Colome, C., Artuch, R., Vilaseca, M.A., Sierra, C., Brandi, N., Cambra, F.J., Lambruschini, N. and Campistol, J. (2002) Clin. Biochem. 35, 81–84.
[22] Mattila, P., Lehtonen, M. and Kumpulainen, J. (2000) J. Agric. Food. Chem. 48, 1229–1233.
[23] Weber, C., Bysted, A. and Hllmer, G. (1997) Int. J. Vitam. Nutr. Res. 67, 123–129.
[24] Liu, S.S. (1997) Biosci. Rep. 17, 259–272.
[25] Turrens, J.F., Alexandre, A. and Lehninger, A.L. (1985) Arch. Biochem. Biophys. 237, 408–414.
[26] Turrens, J.F. (2003) J. Physiol. 552, 335–344.
[27] Åberg, F., Appelkvist, E.L., Dallner, G. and Ernster, L. (1992) Arch. Biochem. Biophys. 295, 230–234.
[28] Tramer, F., Rocco, F., Micali, F., Sandri, G. and Panfili, E. (1998) Biol. Reprod. 59, 753–758.
[29] Harris, A.L. (2002) Nat. Rev. Cancer 2, 38–47.
[30] Hopkins, S.R., Bogaard, H.J., Niizeki, K., Yamaya, Y., Ziegler, M.G. and Wagner, P.D. (2003) J. Physiol. 550, 605–616.
[31] Stroka, D.M., Burkhardt, T., Desbaillets, I., Wenger, R.H., Neil, D.A., Bauer, C., Gassmann, M. and Candinas, D. (2001) FASEB J. 15, 2445–2453.