Free Rad. Res., Vol. 29, pp. 85–92
Reprints available directly from the publisher
Photocopying permitted by license only

Effect of Combined Coenzyme Q10 and d-α-Tocopheryl Acetate Supplementation on Exercise-Induced Lipid Peroxidation and Muscular Damage: a Placebo-Controlled Double-Blind Study in Marathon Runners

JARI KAIKKONEN, LOTTA KOSONEN, KRISTIINA NYYSSÖNEN, ELINA PORKKALA-SARATAHO, RIITTA SALONEN, HEIKKI KORPELA and JUKKA T. SALONEN*

Research Institute of Public Health and the Department of Community Health and General Practice, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland

Accepted by Prof. B. Halliwell

(Received 15 May 1997; In revised form 6 March 1998)

To test the effects of combined coenzyme Q10 (Q10) and d-α-tocopheryl acetate supplementation on exercise-induced oxidative stress and muscular damage we conducted a double-blind study in 37 moderately trained male marathon runners. These were randomly allocated to receive either an antioxidant cocktail: 90 mg of Q10 and 13.5 mg of d-α-tocopheryl acetate daily (18 men) or placebo (19 men) for three weeks before a marathon (42 km) run. Just before the run, plasma Q10 was 282% ($p < 0.0001$) and plasma vitamin E 16% ($p < 0.007$) higher in the supplemented group, than in the placebo group. Also the proportion of plasma ubiquinol of total Q10, an indication of plasma redox status *in vivo*, was significantly higher in the supplemented group. Furthermore, the susceptibility of the VLDL + LDL fraction, to copper-induced oxidation, was significantly reduced in the supplemented group, compared to the placebo group. The exercise increased lipid peroxidation significantly in both study groups, as assessed by the elevated proportion LDL^- of LDL and the increased susceptibility of lipoproteins to copper induced oxidation. However, the supplementation had no effect on lipid peroxidation or on the muscular damage (increase in serum creatine kinase activity or in plasma lactate levels) induced by exhaustive exercise. Plasma ascorbate, Q10, whole blood glutathione and serum uric acid concentrations increased during the exercise, elevating significantly the TRAP value of plasma by 10.3% and the proportion of plasma ubiquinol of total Q10 by 4.9%. These results suggest that even though exercise increases plasma lipid peroxidation, it also elevates the antioxidative capacity of plasma, as assessed by the increased plasma TRAP and the proportion of $Q10H_2$ of total Q10. However, prior supplementation with small doses of Q10 and d-α-tocopheryl acetate neither attenuates the oxidation of lipoproteins nor muscular damage induced by exhaustive exercise such as encountered in a marathon run.

Keywords: α-tocopherol, coenzyme Q10, exhaustive exercise, lipid peroxidation, muscular damage, oxidative stress

*Corresponding author. Fax: 358-17-162936. E-mail: jukka.salonen@uku.fi.

INTRODUCTION

Strenuous exercise has been suggested to increase oxidative stress and to decrease oxidation resistance in the human body.[1] In addition to being an important part of the aerobic energy producing shuttle in the mitochondria, endogenous coenzyme Q10 (Q10) is thought to be a major plasma antioxidant.[2] The effect of Q10 supplementation on exercise-induced muscle damage and oxidative stress has been investigated in rats and in humans, but the existing data are sparse and inconsistent. Furthermore, in previous studies, the number of subjects and the scale of performed measurements have been small. For example, intravenous Q10 supplementation has diminished creatine kinase and lactate dehydrogenase activities following downhill running in rats,[3] but similar effects were not observed in humans following oral supplementation and an exhaustive cycling session.[4] Furthermore, daily oral Q10 supplementation did not reduce serum lipid peroxidation (TBARS) in trained cyclists.[5] The aim of the present study was to obtain confirmation of whether exercise-induced oxidative stress or muscular damage can be decreased by oral Q10 and low dose vitamin E supplementation. In addition, we investigated both the effect of antioxidant supplementation and exercise on the proportion of plasma $Q10H_2$ (ubiquinol) of total Q10, used as an indication of plasma redox status *in vivo*. To our knowledge, the present study is the first placebo-controlled double-blind clinical trial to assess the effect of moderate Q10 and minor vitamin E supplementation on the electrochemically measured proportion of plasma $Q10H_2$ of total Q10 as well as on the combined plasma VLDL + LDL fraction during exercise in moderately trained, healthy subjects.

MATERIALS AND METHODS

Description of Subjects

This randomized double-blinded placebo-controlled study was carried out in connection with the Helsinki City Marathon in July 1995. Thirty-eight healthy moderately trained non-smoking male runners, who were not regular users of antioxidants, acetylsalicylic acid, or other anti-inflammatory analgesics were recruited via newspaper advertisements from Finland. These were randomly allocated to either the antioxidant cocktail group, 3×30 mg of Q10 and 3×4.5 mg of d-α-tocopheryl acetate daily (18 men), or placebo group (19 men) for three weeks before the marathon run. A baseline blood sample was taken just before the run and the second sample taken as soon as possible after the competition. Diet was not restricted before the run, but during the exercise only antioxidant-free beverage was allowed. Three subjects interrupted the competition after running 18, 23 and 34 km, but they were included in the study. One subject in the placebo group had to receive an intravenous infusion after the run and was omitted. Thus, thirty-seven subjects (aged 40 ± 7 years, BMI 23.6 ± 2.1, mean $\pm$ sd) were included in the statistical analysis. The running time of the subjects was 3 h 58 min $\pm$ 31 min (mean $\pm$ sd, $n = 34$) the range being 3 h 9 min to 5 h 21 min. The running time of the interrupters varied from 1 h 35 min to 3 h 38 min. All subjects provided a written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

Analytical Procedures

Samples for measurements in batches were frozen on dry ice (−80°C) immediately after plasma separation.

Thawed EDTA plasma for plasma total Q10 determination was prehandled according to Grossi *et al.*[6] using hexane extraction, but instead of solid-phase extraction cartridges, we used 100 mg of silica powder (Varian, Harbor City, CA) in glass tubes (10 ml) and 50 mg of C-18 powder (Varian) in polypropylene tubes (1 ml) to remove impurities. Otherwise the measurement was carried out as described previously.[7] Proportion of plasma ubiquinol of total Q10 was determined by a chromatographic method,

modified from that presented by Finckh *et al.* (1994).[8] Coenzyme Q7 (Sigma, St. Louis, MO) was used as an internal standard and frozen EDTA plasma pool as a secondary Q10 standard. The total Q10 concentration of the plasma pool was determined with a method described previously.[7] EDTA plasma samples were thawed, extracted (at +4°C) and analyzed one at a time to ensure as minimal sample oxidation as possible during the sample preparation. Q10 concentrations were measured using a Coulochem 5200 A electrochemical detector (ESA, Bedford, MA) with a model 5020 guard cell after the column for reducing quinones to quinols, and a model 5011 analytical cell for oxidizing quinols back to quinones. Ubiquinol and ubiquinone were separated with an HPLC system consisting of an HPLC Pump 420 (Kontron Instruments, Milan, Italy), a Model 7125 sample injector (Rheodyne, Cotati, CA) with a 20 µl loop, the Personal Chromatograph software (System Gold, Beckman, San Ramon, CA) and a column (LiChroCART, 125 × 4 mm, Merck, Darmstadt, Germany). The eluent was 13.4 mM lithium perchlorate in methanol: ethanol: 2-propanol (880/240/100, v/v) at a flow rate of 1.2 ml/min. The within-batch CV of a frozen non-supplemented plasma pool was 3.2% for plasma total Q10 and 2.2% for proportion of $Q10H_2$ of total Q10 ($n = 9$). The between-batch CV was 3.0% for plasma total Q10 and 1.4% for proportion of $Q10H_2$ of total Q10 ($n = 7$).

Total Glutathione (GSH) of whole blood was determined by an enzymatic recycling reaction.[9] Sulfosalicylic acid (Merck, Darmstadt, Germany) was used to precipitate proteins and to stabilize fresh EDTA blood samples. Stabilized samples were kept at +4°C until determined. Production of 5-thio-2-nitrobenzoic acid was measured photometrically at 414 nm in an iEMS microtiter plate reader (Labsystems, Helsinki, Finland).

Plasma ascorbate and α-tocopherol were measured from thawed samples by chromatographic methods.[10,11]

Blood hemoglobin and hematocrit were measured with a blood cell counter (Cell-Dyn 610, Mountain View, CA).

Plasma total antioxidative capacity (TRAP) was measured from thawed EDTA plasma as previously described,[12] using Bio-Orbit 1251 luminometer (Turku, Finland).

Copper-mediated VLDL + LDL oxidation was assessed in isolated VLDL + LDL fraction using sample handling and methods presented previously,[11] except that fresh EDTA plasma samples were frozen at −80°C in 0.6% sucrose (BDH, Poole, England). VLDL + LDL fraction was isolated from thawed sucrose plasma using ultracentrifugation. For copper-induced oxidation, EDTA was removed chromatographically using PD-10 columns (Pharmacia, Uppsala, Sweden). VLDL + LDL was diluted with oxygen-saturated PBS to a protein concentration of 0.05 mg/ml. Formation of conjugated dienes was started by adding 33.5 µl of 100 µM copper chloride (Merck) (final concentration 1.65 µM) to 2 ml of diluted VLDL + LDL fraction and the reaction was assessed spectrophotometrically at 234 nm. Lagtime to the maximum oxidation rate, the maximum oxidation rate (maximum slope) and the oxidation susceptibility (maximum slope/lagtime) were determined by Pharmacia LKB Biochrom 4060 spectrophotometer (Uppsala, Sweden).

Electronegatively charged LDL (LDL^-) was measured from thawed EDTA plasma samples using ion exchange chromatography.[13]

Serum total cholesterol and uric acid were measured enzymatically (Kone Instruments, Finland). Also serum creatine kinase activity and thawed samples for plasma lactate were determined enzymatically (Boehringer Mannheim, Mannheim, Germany).

Statistical Analysis

Wilcoxon rank sum test for independent samples was used to compare heterogeneity in baseline values and in changes between the groups. *t*-test for paired samples and Wilcoxon matched-pairs signed rank test were used to analyse change within subjects. The change in plasma volume during the exercise was taken into account by

correcting the values received after the run as suggested by Dill and Costill.[14] Ninety-five percent confidence intervals were computed based on *t*-distribution.

RESULTS

Effect of Exercise on Lipid Peroxidation and on Plasma Antioxidative Capacity

The effect of exercise was to increase the percentage of LDL^- present in LDL by 22.4% (95% CI, 16.8–27.9%) and to increase lipoprotein susceptibility to copper-induced oxidation by 11.4% (95% CI, 6.5–16.2%) (Table I), reflecting elevated plasma lipid peroxidation. The exhaustive exercise increased significantly both serum creatine kinase by 137% (95% CI, 113–162%) and plasma lactate by 31% (95% CI, 18–44%). On the other hand, at the same time there was an increase in plasma ascorbate, total Q10, whole blood glutathione and serum uric acid concentrations resulted in a 10.3% (95% CI, 6.5–14.1%) elevation in the TRAP value of plasma, and a 4.9% (95% CI, 3.2–6.5%) increase in the proportion of plasma $Q10H_2$ of total Q10, indicating increased plasma antioxidative capacity.

Effect of Supplementation on Lipid Peroxidation and on Plasma Antioxidative Capacity

After the three-week supplementation the plasma Q10 concentration was 282% (95% CI, 230–333%) and the plasma vitamin E 16% (95% CI, 4–29%) higher in the supplemented group compared with the placebo group. Also the proportion of plasma ubiquinol of total Q10, an indication of plasma redox status *in vivo*, was 6.9% (95% CI, 3.6–10.2%) higher in the supplemented group. In addition, the lipoprotein susceptibility to copper-induced oxidation, was 17% (95% CI, 3–31%) lower in the supplemented group, compared with the placebo group.

TABLE I Values (mean ± sd) measured before and after running the marathon

	Mean ± sd ($n = 37$)		*p* for change**	
	Before	After	*t*-test	Wilcoxon
Hemoglobin (g/l)	148 ± 9	153 ± 10	< 0.001	< 0.0001
Hematocrit	0.44 ± 0.03	0.46 ± 0.03	< 0.001	< 0.001
Plasma lactate (mmol/l)*	1.34 ± 0.34	1.76 ± 0.58	< 0.001	< 0.0001
Serum creatine kinase (U/l)*	135 ± 56	319 ± 126	< 0.001	< 0.0001
Serum cholesterol (mmol/l)*	5.45 ± 0.86	5.39 ± 0.82	0.134	0.163
VLDL + LDL oxidation by copper:				
Lagtime to oxidation (min)	79 ± 10	79 ± 10	0.524	0.614
Maximum slope (mabs/min)	10.4 ± 2.2	11.7 ± 1.9	< 0.001	< 0.0001
Oxidation susceptibility (μabs/min^2)	135 ± 32	150 ± 32	< 0.001	< 0.001
LDL^- (%)	11.3 ± 3.2	13.9 ± 3.5	< 0.001	< 0.0001
Plasma TRAP (μmol/l)*	1366 ± 181	1507 ± 229	< 0.001	< 0.0001
Plasma Q10 (μmol/l)*	1.96 ± 1.34	2.03 ± 1.36	0.293	0.040
Plasma Q10 (mmol/mol chol.)	0.36 ± 0.25	0.38 ± 0.24	0.131	0.003
Proportion of $Q10H_2$ of total Q10 (%)	82.0 ± 4.7	86.0 ± 3.5 ($n = 36$)	< 0.001	0.0001
Plasma α-tocopherol (μmol/l)*	31.1 ± 5.9	30.4 ± 5.4	0.031	0.017
Plasma α-tocopherol (mmol/mol chol.)	5.73 ± 0.84	5.67 ± 0.75	0.272	0.712
Blood total GSH (μmol/l)*	817 ± 103	842 ± 114	0.046	0.069
Serum uric acid (μmol/l)*	283 ± 42	324 ± 50	< 0.001	< 0.0001
Plasma ascorbate (μmol/l)*	89.2 ± 25.3	102.8 ± 33.3	< 0.001	< 0.001

* Value obtained after the run is corrected for the change of hematocrit and hemoglobin as suggested by Dill and Costill.[14]
** Changes within group were tested with *t*-test for paired samples and with Wilcoxon matched-pairs signed rank test. Plasma TRAP, total antioxidative capacity of plasma; LDL^- electronegatively charged LDL; oxidation susceptibility, maximum slope/lagtime.

TABLE II Baseline characteristics and changes (mean ± sd) in different variables measured before and after running the marathon

	CoQ10 + α-tocopherol ($n = 18$)		Placebo ($n = 19$)		p for difference**	
	Baseline	Change	Baseline	Change	Baseline	Change
Hemoglobin (g/l)	148 ± 10	6 ± 6	148 ± 8	4 ± 5	0.831	0.593
Hematocrit	0.44 ± 0.03	0.01 ± 0.02	0.44 ± 0.02	0.01 ± 0.02	0.866	0.963
Plasma lactate (mmol/l)*	1.29 ± 0.41	0.58 ± 0.59	1.39 ± 0.25	0.27 ± 0.41	0.162	0.078
Serum creatine kinase (U/l)*	127 ± 41	173 ± 98	141 ± 68	196 ± 99	0.796	0.466
Serum cholesterol (mmol/l)*	5.39 ± 0.65	−0.08 ± 0.31	5.50 ± 1.04	−0.05 ± 0.18	0.927	0.903
VLDL + LDL oxidation by copper:						
Lagtime to oxidation (min)	83 ± 11	1 ± 5	75 ± 7	1 ± 5	0.012	0.641
Maximum slope (mabs/min)	9.9 ± 2.4	1.4 ± 1.3	10.9 ± 1.8	1.0 ± 1.1	0.207	0.429
Oxidation susceptibility (μabs/min^2)	122 ± 32	18 ± 18	147 ± 27	13 ± 19	0.045	0.447
LDL$^-$ (%)	11.7 ± 2.9	2.9 ± 2.0	11.0 ± 3.5	2.2 ± 1.8	0.331	0.248
Plasma TRAP (μmol/l)*	1391 ± 174	129 ± 183	1342 ± 189	152 ± 127	0.605	0.671
Plasma Q10 (μmol/l)*	3.16 ± 0.89	0.04 ± 0.55	0.83 ± 0.20	0.09 ± 0.08	< 0.0001	0.584
Plasma Q10 (mmol/mol chol.)	0.59 ± 0.15	0.01 ± 0.09	0.15 ± 0.04	0.02 ± 0.01	< 0.0001	0.952
Proportion of $Q10H_2$ of total Q10 (%)	84.7 ± 4.1	3.1 ± 4.0	79.2 ± 3.6 ($n = 18$)	4.9 ± 3.8 ($n = 18$)	< 0.001	0.114
Plasma α-tocopherol (μmol/l)*	33.5 ± 4.3	−1.1 ± 2.1	28.8 ± 6.3	−0.3 ± 1.6	0.007	0.202
Plasma α-tocopherol (mmol/mol chol.)	6.26 ± 0.82	−0.13 ± 0.40	5.23 ± 0.48	0.01 ± 0.26	< 0.001	0.378
Blood total GSH (μmol/l)*	801 ± 113	26 ± 85	831 ± 93	26 ± 69	0.288	0.927
Serum uric acid (μmol/l)*	280 ± 40	37 ± 36	286 ± 45	44 ± 36	0.616	0.523
Plasma ascorbate (μmol/l)*	89.5 ± 30.6	12.9 ± 15.8	89.0 ± 19.9	14.2 ± 19.3	0.638	0.761

*Value obtained after the run is corrected for the change of hematocrit and hemoglobin as suggested by Dill and Costill.[14]
**Differences at baseline and in change between groups were tested with Wilcoxon rank sum test for independent samples.
LDL$^-$, electronegatively charged LDL; oxidation susceptibility, maximum slope/lagtime.

However, the supplementation had no effect on the exercise-induced oxidative stress (increase in electronegatively charged LDL, parameters of copper-induced VLDL + LDL oxidation) (Table II). The supplementation neither spared water soluble antioxidants (ascorbate and uric acid in plasma or total glutathione in whole blood) during the run nor had any effect on the plasma TRAP. Furthermore, there was no difference in the increase of muscular damage (serum creatine kinase activity) or muscle metabolites (plasma lactate) between the supplemented and placebo groups (Table II).

DISCUSSION

In the present study, the oxidative stress increased during the exercise, whether this was assessed by parameters of copper induced oxidation or by increase of LDL$^-$ in the LDL fraction (Table I). As noted in previous studies, the human body can adapt to the oxidative stress encountered during exhaustive exercise by increasing activities of important defense enzymes, such as glutathione peroxidase, superoxide dismutase and catalase and by elevating the amount of different antioxidants in plasma.[15] Also in the present study, serum uric acid, plasma ascorbate, plasma Q10 and blood total GSH concentrations increased during the exercise, elevating not only plasma total antioxidative capacity, but also the proportion of plasma $Q10H_2$ of total Q10. In previous studies, the proportion of $Q10H_2$ of total Q10, an indication of plasma redox status *in vivo*, has been decreased in several pathological conditions such as hyperlipidemia and coronary artery disease.[16,17] However, no

exercise-induced change in this ratio has been observed with UV-detection methods.[18,19] Thus, the present study is the first trial to suggest that exercise can elevate this ratio, possibly reflecting the beneficial effects of exercise on the circulation. However, it is possible that the elevation of the concentrations of different antioxidants in plasma may be due to tissue damage, as well as increased synthesis or active release from the tissues. The aim of the present study was to provide additional information about whether the exercise-induced oxidative stress and/or muscular damage could be decreased by oral Q10 and low dose vitamin E supplementation.

Even though vitamin E and Q10 may have synergistic effects on antioxidative function,[20] our results suggest that 90 mg of Q10 daily in combination with a low vitamin E dose neither reduces the exercise-induced plasma lipid peroxidation nor elevates the total antioxidative capacity of plasma in healthy, moderately trained subjects. Muscular damage and exhaustion due to exercise were assessed by CK and lactate measurements. The changes in these parameters were also not affected by the antioxidant supplementation. These results are in accordance with small-scale supplementation studies in healthy trained or untrained men with similar daily doses.[4,5] Braun and coworkers[5] measured the exercise-induced change in serum malondialdehyde (TBARS) concentration before and after a two-month Q10 supplementation (100 mg daily) in ten male bicycle racers. As compared with the placebo group ($n = 5$), no significant change in the increase of TBARS concentration was detected in the supplemented group ($n = 5$). Zuliani *et al.*[4] studied the effect of one-month Q10 supplementation (100 mg daily) on the change of different parameters induced by prolonged work on an ergometer bicycle, measured before, at the end of aerobic work, at the end of exhaustive work, and after 30 and 60 min of the recovery phase. Their finding was that the supplementation had no influence on the change of free glycerol, lactate, glucose insulin or CK concentrations measured at these different time points in 12 healthy untrained men. Also some harmful effects of Q10 supplementation have been reported. Malm and coworkers[21] found that Q10 supplementation (120 mg daily) in healthy male subjects possibly caused cellular damage during intense short-term anaerobic exercise (a cycling session), as reflected as an increase in plasma CK activity in the supplemented group ($n = 9$), compared with the placebo group ($n = 6$).

On the other hand, some findings suggest that oral Q10 supplementation (90–100 mg daily for one to six months) might increase the physical working capacity (heart function, maximal oxygen consumption) in healthy subjects.[22–25] However, Porter and coworkers[26] stated that Q10 supplementation with doses, as high as 150 mg daily for two months, had no effect on the working capacity of middle-aged, untrained men. Also Laaksonen and coworkers[27] reported in a double-blind placebo-controlled study that 120 mg of oral Q10 daily for 6 weeks neither increased working capacity nor had any effect on lipid peroxidation (serum malondialdehyde) in trained young or older men ($n = 9$). The variability in the results of different exercise studies may be due to the ability of supplemented Q10 to act as an antioxidant, but at the same time to elevate maximal oxygen consumption, which probably leads to an increased free radical flux in the body. Also differences in subjects and in the bioavailability of the supplements used may account for some of the variation in the results. It may be that oral Q10 supplementation (90–100 mg daily) has beneficial antioxidative or protective effects during exercise only in persons with pathological conditions leading to low Q10 status or when there is an elevated requirement of Q10.[28,29] Alternatively intravenous administration of massive doses might also be capable of reducing muscular damage, as observed in a previous rat study.[3] Due to its limited absorption,[30] the extensive concentration gradient in Q10 between plasma and tissues[31] and possible

down-regulated synthesis, it may be difficult to elevate tissue (muscle) Q10 concentrations by oral supplementation in healthy subjects. The supplementation time was only three weeks in our study, but that should be enough to elevate the plasma lipoprotein concentration to its maximum level. However, to increase the tissue (muscle) Q10 concentrations by oral dosing, both larger doses and longer supplementation times may be needed.

Even though no response to antioxidants was observed during the exercise, our results suggest that combined moderate Q10 and minor vitamin E supplementation may elevate the proportion of plasma $Q10H_2$ of total Q10 and also increase the oxidation resistance of isolated plasma lipoproteins. However, we emphasize that for practical reasons, no baseline measurements of oxidation susceptibility were carried out before starting the antioxidant supplementation. Furthermore, our previous finding in smoking men suggested that 90 mg of Q10 daily is not enough to increase the oxidation resistance of isolated lipoproteins exposed to *in vitro* high radical flux.[7] On the other hand, even a one week supplementation with 90 mg of Q10 daily tended to elevate the proportion of plasma $Q10H_2$ of total Q10 in our previous study, possibly indicating an attenuated plasma lipid peroxidation *in vivo*.[32] That result was confirmed in the present study.

In conclusion, in addition to increasing the TRAP value of plasma, exercise elevates the proportion of plasma $Q10H_2$ of total Q10, probably a reflection of its beneficial effects on circulation. Oral Q10 supplementation of 90 mg daily, alone or combined with low doses of α-tocopherol might elevate the proportion of plasma $Q10H_2$ of total Q10 and improve the oxidation resistance of LDL + VLDL in sedentary conditions, but it does not appear to attenuate either the oxidation of lipoproteins or the muscular damage induced by exhaustive exercise in healthy, moderately trained subjects. This observation should be retested in further larger trials with higher doses of antioxidants.

Acknowledgements

This study was supported by the Ministry of Education of Finland, Yrjö Jahnsson foundation, Finland and Pharma Nord, Finland. We thank our laboratory staff for performing the blood and plasma analyses. The Q10 supplements used were from Pharma Nord, Denmark.

References

[1] M.M. Kanter (1994). Free radicals, exercise, and antioxidant supplementation. *International Journal of Sport Nutrition*, **4**, 205–220.

[2] R.E. Beyer (1992). An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. *Biochemistry and Cell Biology*, **70**, 390–403.

[3] Y. Shimomura, M. Suzuki, S. Sugiyama, Y. Hanaki and T. Ozawa (1991). Protective effect of coenzyme Q10 on exercise-induced muscular injury. *Biochemical and Biophysical Research Communications*, **176**, 349–355.

[4] U. Zuliani, A. Bonetti, M. Campana, G. Cerioli, F. Solito and A. Novarini (1989). The influence of ubiquinone (CoQ10) on the metabolic response to work. *The Journal of Sports Medicine and Physical Fitness*, **29**, 57–62.

[5] B. Braun, P.M. Clarkson. P.S. Freedson and R.L. Kohl (1991). Effects of coenzyme Q10 supplementation on exercise performance, VO_2max and lipid peroxidation in trained cyclists. *International Journal of Sport Nutrition*, **1**, 353–365.

[6] G. Grossi, A.M. Bargossi, P.L. Fiorella and S. Piazzi (1992). Improved high-performance liquid chromatographic method for the determination of coenzyme Q10 in plasma. *Journal of Chromatography*, **593**, 217–226.

[7] J. Kaikkonen, K. Nyyssönen, E. Porkkala-Sarataho, H.E. Poulsen, T. Metsä-Ketelä, M. Hayn, R. Salonen and J.T. Salonen (1997). Effect of oral coenzyme Q10 supplementation on the oxidation resistance of human VLDL + LDL fraction: absorption and antioxidative properties of oil and granule-based preparations. *Free Radical Biology & Medicine*, **22**, 1195–1202.

[8] B. Finckh, A. Kontush, J. Commentz, C. Hubner, M. Burdelski and A. Kohlschutter (1995). Monitoring of ubiquinol-10, ubiquinone-10, carotenoids, and tocopherols in neonatal plasma microsamples using high-performance liquid chromatography with coulometric electrochemical detection. *Analytical Biochemistry*, **232**, 210–216.

[9] M.A. Baker, G.J. Cerniglia and A. Zaman (1990). Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Analytical Biochemistry*, **190**, 360–365.

[10] K. Nyyssönen, S. Pikkarainen, M.T. Parviainen, K. Heinonen and I. Mononen (1988). Quantitative estimation of dehydroascorbic acid and ascorbic acid by high-performance liquid chromatography – application to human milk, plasma, and leukocytes. *Journal of Liquid Chromatography*, **11**, 1717–1728.

[11] E. Porkkala-Sarataho, K. Nyyssönen and J.T. Salonen (1996). Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in

non-vitamin E supplemented men. *Atherosclerosis*, **124**, 83–94.

[12] M. Ristola, T. Metsä-Ketelä, M. Leirisalo-Repo and H. Repo (1992). Oxygen radical production and trapping in subjects with previous yersinia infection. *Inflammation*, **16**, 273–283.

[13] K. Nyyssönen, J. Kaikkonen and J.T. Salonen (1996). Characterization and determinants of an electronegatively charged low-density lipoprotein in human plasma. *The Scandinavian Journal of Clinical & Laboratory Investigation*, **56**, 681–689.

[14] D.B. Dill and D.L. Costill (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, **37**, 247–248.

[15] J.C. Dekkers, L.J.P. van Doornen and H.C.G. Kemper (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. *Sports Medicine*, **21**(3), 213–238.

[16] J. Lagendijk, J.B. Ubbink, R. Delport, W.J. Vermaak and J.A. Human (1997). Ubiquinol/ubiquinone ratio as marker of oxidative stress in coronary artery disease. *Research Communications in Molecular Pathology and Pharmacology*, **95**, 11–20.

[17] A. Kontush, A. Reich, K. Baum, T. Spranger, B. Finckh, A. Kohlschutter and U. Beisiegel (1997). Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia. *Atherosclerosis*, **129**, 119–126.

[18] T.J. Vasankari, U.M. Kujala, T.M. Vasankari, T. Vuorimaa and M. Ahotupa (1997). Effects of acute prolonged exercise on serum and LDL oxidation and antioxidant defences. *Free Radical Biology & Medicine*, **22**, 509–513.

[19] T.J. Vasankari, U.M. Kujala, T.M. Vasankari, T. Vuorimaa and M. Ahotupa (1997). Increased serum low-density-lipoprotein antioxidant potential after antioxidant supplementation in endurance athletes. *American Journal of Clinical Nutrition*, **65**, 1052–1056.

[20] S.R. Thomas, J. Neuzil and R. Stocker (1996). Cosupplementation with coenzyme Q prevents the prooxidant effect of α-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation *Arteriosclerosis, Thrombosis and Vascular Biology*, **16**, 687–696.

[21] C. Malm, M. Svensson, B. Sjöberg, B. Ekblom and B. Sjödin (1996). Supplementation with ubiquinone-10 causes cellular damage during intense exercise. *Acta Physiologica Scandinavica*, **157**, 511–512.

[22] E. Amadio, R. Palermo, G. Peloni and G.P. Littarru (1991). Effect of CoQ10 administration on VO_2max and diastolic function in athletes. In *Biomedical and Clinical Aspects of Coenzyme Q* (eds. K. Folkers, G.P. Littarru and T. Yamagami), Elsevier Science Publishers, Amsterdam, New York, Oxford, pp. 525–533.

[23] H. Yamabe and H. Fukuzaki (1991). The beneficial effect of coenzyme Q10 on the impaired aerobic function in middle aged women without organic disease. In *Biomedical and Clinical Aspects of Coenzyme Q* (eds. K. Folkers, G.P. Littarru and T. Yamagami), Elsevier Science Publishers, Amsterdam, New York, Oxford, pp. 535–549.

[24] P. Zeppilli, B. Merlino, A. de Luca, V. Palmieri, C. Santini, R. Vannicelli, M. la Rosa Gangi, R. Caccese, S. Cameli, S. Servidei, E. Ricci, G. Silvestri, S. Lippa, A. Oradei and G.P. Littarru (1991). Influence of coenzyme-Q10 on physical work capacity in athletes, sedentary people and patients with mitochondrial disease. In *Biomedical and Clinical Aspects of Coenzyme Q* (eds. K. Folkers, G.P. Littarru and T. Yamagami), Elsevier Science Publishers, Amsterdam, New York, Oxford, pp. 541–545.

[25] T. Ylikoski, J. Piirainen, O. Hanninen and J. Penttinen (1997). The effect of coenzyme Q10 on the exercise performance of cross-country skiers. *Molecular Aspects of Medicine*, **18**, 283–290.

[26] D.A. Porter, D.L. Costill, J.J. Zachwieja, K. Krzeminski, W.J. Fink, E. Wagner and K. Folkers (1995). The effect of oral coenzyme Q10 on the exercise tolerance of middle-aged, untrained men. *International Journal of Sports Medicine*, **16**, 421–427.

[27] R. Laaksonen, M. Fogelholm, J.J. Himberg, J. Laakso and Y. Salorinne (1995). Ubiquinone supplementation and exercise capacity in trained young and older men. *European Journal of Applied Physiology and Occupational Physiology*, **72**, 95–100.

[28] S. Ogasahara, Y. Nishikawa, S. Yorifuji, F. Soga, Y. Nakamura, M. Takahashi, S. Hashimoto, N. Kono and S. Tarui (1986). Treatment of Kearns-Sayre syndrome with Coenzyme Q10. *Neurology*, **36**, 45–53.

[29] S. Fujimoto, N. Kurihara, K. Hirata and T. Takeda (1993). Effects of coenzyme Q10 administration on pulmonary function and exercise performance in patients with chronic lung disease. *Clinical Investigator*, **71**(8 Suppl.), 162–166.

[30] P.W. Lucker, N. Wetzelsberger, G. Hennings and D. Rehn (1984). Pharmacokinetics of coenzyme ubidecarenone in healthy volunteers. In *Biomedical and Clinical Aspects of Coenzyme Q*, Vol. 4 (eds. K. Folkers and Y. Yamamura), Elsevier Science Publishers B.V., Amsterdam, pp. 143–151.

[31] R. Laaksonen, A. Ruhimäki, J. Laitila, K. Mårtensson, M.J. Tikkanen and J.J. Himberg (1995). Serum and muscle tissue ubiquinone levels in healthy subjects. *Journal of Laboratory and Clinical Medicine*, **125**, 517–521.

[32] J. Kaikkonen, K. Nyyssönen, T.P. Tuomainen, E. Porkkala-Sarataho and J.T. Salonen. Interaction in absorption and in antioxidative efficiency between coenzyme Q10 and vitamin E in mildly hypercholesterolemic subjects: a placebo-controlled, clinical supplementation study. (Submitted for publication).