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DOI: 10.1373/clinchem.2005.054353

Determination of Coenzyme Q₁₀ Status in Blood Mononuclear Cells, Skeletal Muscle, and Plasma by HPLC with Di-Propoxy-Coenzyme Q₁₀ as an Internal Standard,

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Coenzyme Q₁₀ (CoQ₁₀), the predominant ubiquinone species in humans, functions as an electron carrier in the mitochondrial electron transport chain (ETC) and as an intracellular antioxidant (1). Although primary CoQ₁₀ deficiency is rare, a profound deficiency in skeletal muscle CoQ₁₀ has been reported in patients with multisystem mitochondrial encephalomyopathies (2, 3). Cardiovascular disease has been associated with a CoQ₁₀ deficiency (4, 5), and it is becoming increasingly apparent that other groups of patients may become CoQ₁₀ deficient, particularly individuals with ataxia (6) and some patients receiving statins (7).

When assessing tissue CoQ₁₀ status, we have found that the lack of a commercially available nonphysiologic internal standard (IS) is a major difficulty. Although naturally

occurring ubiquinones have been used as ISs in this determination, they are not free from the influence of ubiquinones that might be present in human tissue as the result of dietary contamination (8) or synthesis by microorganisms (9, 10). There is a need, therefore, for an alternative IS that is not influenced by exogenous/endogenous ubiquinones. Di-ethoxy-CoQ₁₀ has been suggested as a nonphysiologic IS to determine CoQ₁₀ (11). In this study we evaluated this IS along with di-propoxy-CoQ₁₀ for their suitability to determine tissue CoQ₁₀. Reference intervals were established for the CoQ₁₀ concentration of skeletal muscle, blood mononuclear cells (MNCs), and plasma. A patient with a suspected CoQ₁₀ deficiency was subsequently identified.

Reference intervals were established for the following: (a), skeletal muscle from 26 patients [mean (SE) age, 24.5 (3.9) years; range, 0.5–59 years; ratio of males to females, 7:6] with no evidence of an ETC deficiency detected in their skeletal muscle biopsies; (b), MNCs from 17 healthy volunteers and 13 disease controls with no clinical evidence of an ETC deficiency [mean (SE) age, 32.6 (2.6) years; range, 1–61 years; ratio of males to females, 7:8]; and (c), plasma from 24 patients [mean (SE) age, 14.3 (2.9) years; range, 1–57 years; ratio of males to females, 2:1] with no clinical evidence of a ETC deficiency.

The correlation between skeletal muscle, MNC, and plasma CoQ₁₀ status was assessed in 2 groups of patients with no clinical or biochemical evidence of an ETC deficiency: Group 1 consisted of 12 patients [mean (SE) age, 13.21 (4.03) years; range, 1–43 years; male/female, 2:1]; plasma was obtained from 10 patients in this group. Group 2 consisted of 14 patients [mean (SE) age, 14.3 (3.7) years; range, 1–57 years; male/female, 4:3]. Correlations between skeletal muscle and MNC CoQ₁₀ status and between skeletal muscle and plasma CoQ₁₀ status were determined with samples from group 1; correlations between MNC and plasma CoQ₁₀ status were determined with samples from groups 1 and 2.

The patient with a suspected CoQ₁₀ deficiency was a 47-year-old female, mentally retarded since birth, ataxic, and with poor vision and hypertrophic cardiomyopathy, in whom evidence of an ETC complex II-III (succinate cytochrome *c* reductase) deficiency [0.015; reference interval, 0.040–0.204 (activity expressed as a ratio to citrate synthase activity to allow for mitochondrial enrichment) (12)] had been detected in skeletal muscle.

MNCs were isolated from 5–10 mL of sodium EDTA-anticoagulated blood within 24 h of venesection by use of the ACCUSPIN™ system–Histopaque®-1077 (Sigma-Aldrich). The MNCs were suspended in phosphate-buffered saline (150 mmol/L NaCl, 150 mmol/L sodium phosphate), pH 7.2 (200 μL per 5 mL of blood), and stored at –70 °C until analysis. During this procedure, plasma was separated from the sodium EDTA-anticoagulated blood and stored at –70 °C until analysis.

Skeletal muscle biopsy homogenates were prepared as described by Heales et al. (12). Protein concentration was determined by the method of Lowry et al. (13). The synthesis of di-ethoxy-CoQ₁₀ was undertaken as de-

scribed by Edlund (11). The synthesis of di-propoxy-CoQ₁₀ was based on the method of Edlund (11), substituting propan-1-ol for ethanol. The concentration of di-propoxy-CoQ₁₀ was estimated based on the molar absorptivity for CoQ₁₀ at 275 nm (14.6×10^3), and the di-propoxy-CoQ₁₀ was diluted in ethanol to give a final concentration of 1.5 $\mu\text{mol/L}$.

Samples were prepared for HPLC analysis of total CoQ₁₀ concentration by the addition of IS (30 μL) to skeletal muscle (50 μL), to MNCs (150 μL), and to plasma (200 μL) to give a final concentration of 150 nmol/L in the reconstituted extract. The ubiquinones (CoQ₁₀ and IS) were extracted by the method of Boitier et al. (14). The extracts were evaporated under N₂ and reconstituted in ethanol (300 μL). HPLC analysis was performed according to the method of Boitier et al. (14).

CoQ₁₀ and di-propoxy-CoQ₁₀ were analyzed at concentrations of 50 $\mu\text{mol/L}$ by mass spectrometry using a Quattro micro triple-quadrupole tandem mass spectrometer operating in both the scan and parent ion modes (15).

We used regression analysis to assess the correlation between ultraviolet absorbance (275 nm) and the concentrations of di-propoxy-CoQ₁₀ and skeletal muscle, MNC, and plasma CoQ₁₀, and between age and the MNC, skeletal muscle, and plasma CoQ₁₀ concentration. The relationship between sex and tissue CoQ₁₀ concentration was assessed by the Mann-Whitney *U*-test. Spearman rank correlation coefficients were calculated to assess the association between the CoQ₁₀ concentrations in skeletal muscle, MNCs, and plasma. A *P* value <0.05 was considered significant.

Analysis of the mass spectrum obtained in scan mode for the di-propoxy-CoQ₁₀ IS demonstrated 1 predominant ion of *m/z* 942 (see Fig. 1B in the Data Supplement that accompanies the online version of this Technical Brief at <http://www.clinchem.org/content/vol51/issue12>). This corresponded with the theoretical mass calculated for the sodium adduct of di-propoxy-CoQ₁₀, [M+Na]. An observed increase in molecular mass of 56 Da in di-propoxy-CoQ₁₀ relative to CoQ₁₀ (see Fig. 1A in the online Data Supplement) would correspond to the formation of the di-propoxy derivative. A small amount of impurities (<5%) was observed in the straight-scan analysis of di-propoxy-CoQ₁₀ (see Fig. 1B in the online Data Supplement). Production analysis of both CoQ₁₀ and di-propoxy-CoQ₁₀ (Fig. 2, A and B, in the online Data Supplement) demonstrated clearly that these impurities were not CoQ₁₀ analogs, but we were unable to confirm their identities. At the concentration of di-propoxy-CoQ₁₀ used in tissue determinations (150 nmol/L), these impurities would be undetected on reversed-phase HPLC. Di-propoxy-CoQ₁₀ is stable during the tissue extraction procedure and can be stored for up to 1 year at -70 °C with no evidence of degradation. Di-ethoxy-CoQ₁₀ was poorly resolved from CoQ₁₀ on reversed-phase HPLC (see Fig. 3 in the online Data Supplement), and no further evaluation of this IS was undertaken. In contrast, di-propoxy-CoQ₁₀ was clearly separated from CoQ₉ and CoQ₁₀ (Fig. 1). The ultraviolet absorbance (275 nm) of

di-propoxy-CoQ₁₀ showed linearity ($r^2 = 0.999$) over the concentration range 0–1000 nmol/L. Use of this IS (500 nmol/L CoQ₁₀ added to skeletal muscle homogenate with an endogenous CoQ₁₀ concentration of 350 nmol/L) gave a mean (SE) recovery of 99.8 (2.9)% ($n = 5$) of CoQ₁₀ in the assay. The intraassay CVs for the assessment of CoQ₁₀ in skeletal muscle, plasma, and MNC samples were 3.4% (mean concentration, 791 nmol/L; $n = 6$), 4.4% (201 nmol/L; $n = 6$), and 2.6% (331 nmol/L; $n = 5$), respectively. The interassay CVs for CoQ₁₀ determination in skeletal muscle, MNCs, and plasma were 3.1% (861 nmol/L; $n = 4$), 3.5% (471 nmol/L; $n = 5$), and 4.5% (760 nmol/L; $n = 4$), respectively, when the di-propoxy-CoQ₁₀ was used as IS. Detection of CoQ₁₀ was linear between 10 and 1000 nmol/L in skeletal muscle ($r^2 = 0.997$), MNCs ($r^2 = 0.995$), and plasma ($r^2 = 0.991$). The limit of detection of CoQ₁₀ was 6 nmol/L for all tissues.

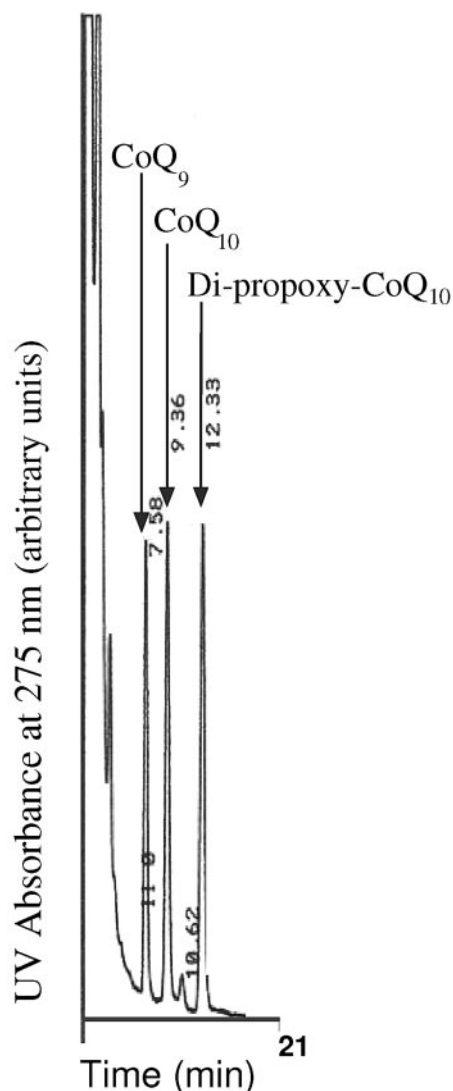


Fig. 1. Reversed-phase HPLC chromatogram of CoQ₉, CoQ₁₀, and di-propoxy-CoQ₁₀.

Retention times of ubiquinones: 7.58 min (CoQ₉), 9.36 min (CoQ₁₀), and 12.33 min (di-propoxy-CoQ₁₀). UV, ultraviolet.

Table 1. Reference intervals for skeletal muscle, MNC, and plasma CoQ₁₀ concentrations.

	CoQ ₁₀ concentration	Units
Skeletal muscle		
Observed range	140–580	pmol/mg of protein
Mean (SD)	241 (95)	pmol/mg of protein
MNCs		
Observed range	37–133	pmol/mg of protein
Mean (SD)	65 (24)	pmol/mg of protein
Plasma		
Observed range	227–1432	nmol/L
Mean (SD)	675 (315)	nmol/L

Reference intervals for skeletal muscle, MNCs, and plasma were established from the observed range of CoQ₁₀ concentrations for these tissues (Table 1). The reference intervals for skeletal muscle and plasma were comparable to those reported by Artuch et al. (16) and Miles et al. (17) for skeletal muscle and plasma, respectively. To our knowledge, there have been no reference intervals for MNC CoQ₁₀ reported by other laboratories. Age and sex had no significant influence on tissue CoQ₁₀ concentrations in the reference population, allowing the effect of these variables to be excluded from the study (results not shown). By comparing the reference intervals, we found evidence of a CoQ₁₀ deficiency in skeletal muscle (33 pmol/mg of protein) and MNCs (20 pmol/mg of protein) in the 47-year-old female patient with low skeletal muscle complex II-III activity.

The decreased CoQ₁₀ status of MNCs and skeletal muscle from this patient suggested that a relationship might exist between the CoQ₁₀ status of these tissues, and this prompted us to assess the relationship between skeletal muscle, MNC, and plasma CoQ₁₀. We found a close association between skeletal muscle and MNC CoQ₁₀ concentrations in the 12 disease control patients and in the CoQ₁₀-deficient patient ($r = 0.89$; $P < 0.02$; $n = 13$). Exclusion of the CoQ₁₀-deficient patient from this correlation did not significantly alter this relationship ($r = 0.86$; $P < 0.02$; $n = 12$). We found no correlation between skeletal muscle and plasma CoQ₁₀ concentrations ($r = 0.015$; $n = 10$) or between MNC and plasma CoQ₁₀ concentrations ($r = 0.21$; $n = 24$).

In conclusion, we have synthesized a di-propoxy-CoQ₁₀ IS that can be used in CoQ₁₀ assessment in MNCs, skeletal muscle, and plasma, allowing precision and a good recovery. This IS enabled the establishment of reference intervals for the CoQ₁₀ concentrations of skeletal muscle, MNCs, and plasma, which has facilitated the identification of a patient with a CoQ₁₀ deficiency.

A.J. Duncan was supported by a grant from the Brain Research Trust (UK) awarded to Dr. S.J.R. Heales. Dr. I.P. Hargreaves is the recipient of an Association of Clinical Biochemists (UK) scholarship award, which also funded part of this work.

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DOI: 10.1373/clinchem.2005.054643

Serum Tartrate-Resistant Acid Phosphatase 5b or Amino-Terminal Propeptide of Type I Procollagen for Monitoring Bisphosphonate Therapy in Postmenopausal Osteoporosis? Matti J. Välimäki¹ and Riitta Tähtelä² (¹ Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; ² Mehiläinen Oy Laboratoriopalvelut, Helsinki, Finland; * address correspondence to this author at: Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland; fax 358-9-47175798, e-mail matti.valimaki@hus.fi)

Bone markers to monitor the efficacy of antiresorptive therapy of osteoporosis are of great value to clinicians. Considerable decreases in markers can be seen within 3 to 6 months after the start of an efficient treatment, with considerable increases in bone mineral density (BMD)