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Biochemical Diagnosis of Coenzyme Q_{10} Deficiency

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Antioxidant · Cholesterol · Coenzyme Q_{10} · Disease · Mitochondria · Muscle · Plasma · Tissue

Abstract
Coenzyme Q_{10} (CoQ_{10}) deficiency appears to have a particularly heterogeneous clinical presentation. However, there appear to be 5 recognisable clinical phenotypes: encephalomyopathy, severe infantile multisystemic disease, nephropathy, cerebellar ataxia, and isolated myopathy. However, although useful, clinical symptoms alone are insufficient for the definitive diagnosis of CoQ_{10} deficiency which relies upon biochemical assessment of tissue CoQ_{10} status. In this article, we review the biochemical methods used in the diagnosis of human CoQ_{10} deficiency and indicate the most appropriate tissues for this evaluation.

Coenzyme Q_{10} (CoQ_{10}) plays an important role in oxidative phosphorylation where it acts as an electron carrier in the mitochondrial electron transport chain (ETC; fig. 1), accepting electrons derived from complex I (NADH ubiquinone reductase; EC 1.6.5.3) and complex II (succinate ubiquinone reductase; EC 1.3.5.1) and transferring them to complex III (ubiquinol cytochrome c reductase; EC 1.10.2.2) [Ernster and Dallner, 1995]. The reduced form of CoQ_{10}, ubiquinol, has an important cellular antioxidant function; it protects membranes and plasma lipoproteins against free radical-induced oxidation [Ernster and Forsmark-Andrée, 1993]. In addition, CoQ_{10} is also involved in DNA replication and repair through its role in pyrimidine synthesis [Lopez-Martin et al., 2007], modulation of apoptosis via its regulation of the mitochondrial permeability transition pore [Cotan et al., 2011], and body temperature regulation via its obligatory cofactor role for the uncoupling proteins [Echtay et al., 2001].

In view of its role as an electron carrier in the ETC and its antioxidant function, a deficit in CoQ_{10} status could conceivably contribute to disease pathophysiology by causing a failure in energy metabolism and compromising cellular antioxidant status.

The first cases of CoQ_{10} deficiency were reported in 1989 by Ogasahara et al. The patients were 2 sisters born to unrelated parents who presented with recurrent rhabdomyolysis associated with seizures and mental retardation. Since this time, a number of patients have been described, and CoQ_{10} deficiency appears to have a particularly heterogeneous clinical presentation. However, there appear to be 5 distinct clinical phenotypes: encephalomyopathy, severe infantile multisystemic disease, nephropa-

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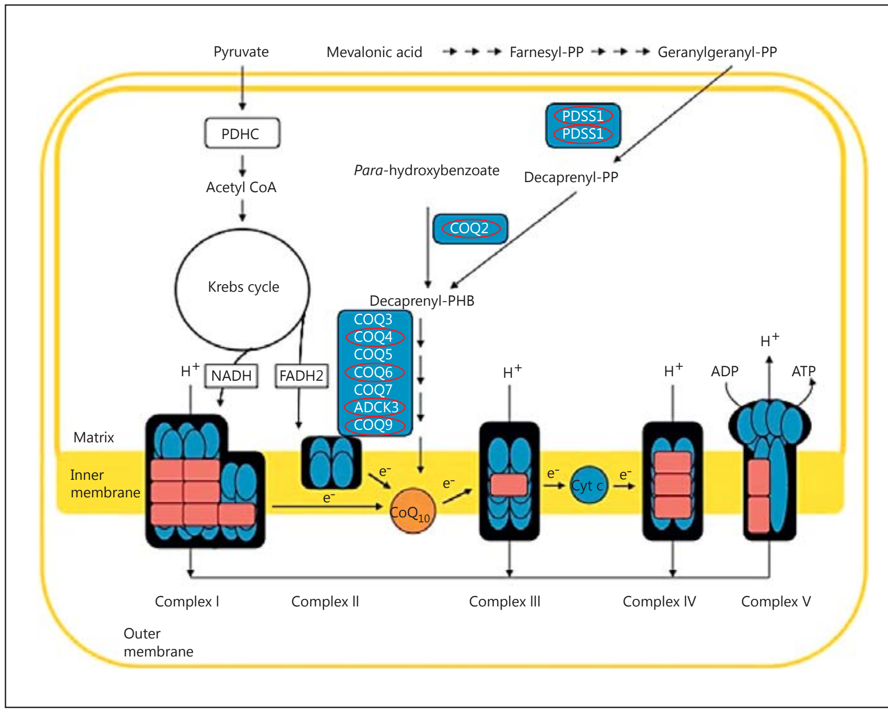

Fig. 1. Structure of mitochondrial electron transport chain, showing the electron carrier function of coenzyme Q_{10} (Q). Red circles indicate proteins with mutations causing CoQ_{10} deficiency; see table 1.

thy, cerebellar ataxia, and isolated myopathy [Emmanuele et al., 2012]. In most cases, the family history suggests an autosomal recessive mode of inheritance. Since 2006, mutations in 7 genes, encoding components closely related with the CoQ_{10} biosynthetic pathway, have been associated with human CoQ_{10} deficiency (table 1). However, in the preponderance of patients with CoQ_{10} deficiency, it has not been possible to identify the underlying genetic cause [Rahman et al., 2012]. The genetic diagnosis of CoQ_{10} deficiency is complicated by the fact that the CoQ_{10} biosynthetic pathway has yet to be fully elucidated in humans, and the possibility arises that the cause of the deficit may result from pathogenic mutations in genes not directly related to CoQ_{10} synthesis [Emmanuele et al., 2012].

The purpose of this article is to critically review the current biochemical methods used in the diagnosis of human CoQ_{10} deficiency and to indicate the most appropriate tissues for this evaluation.

Methods Used to Determine Tissue CoQ_{10} Status

The most common laboratory procedures used for diagnosis of CoQ_{10} deficiency are based on high-pressure liquid chromatography with ultraviolet (HPLC-UV) or electrochemical (HPLC-ED) detection systems. Other procedures, such as tandem-mass spectrometry, have been employed for tissue CoQ_{10} assessment; however, this method will not be discussed further in this article and the reader is referred to the review by Barshop and Gangoiti [2007] which discusses this analytical procedure. Although it is possible to determine the reduced (ubiquinol) and oxidized forms of CoQ_{10} concomitantly by HPLC-ED analysis, diagnostically, the determination of total tissue CoQ_{10} status is sufficiently accurate to detect human CoQ_{10} deficiencies. Simultaneous measurement of both reduced and oxidized forms of CoQ_{10} usually requires a complex pre-analytical management of samples, and chromatographically, this is more complicated than the measurement of total CoQ_{10}. Furthermore, the propensity of ubiquinol to oxidise to CoQ_{10} unless frozen immediately at –80°C may detract from the clinical utility of this determination [Molyneux et al., 2008]. Therefore, the simultaneous assessment of both reduced and oxidised forms of CoQ_{10} is probably more suitable for research purposes rather than for clinical diagnosis. However, in appropriately handled tissue samples the ratio of ubiquinol:CoQ_{10} has been used as a marker of oxidative stress [Niklowitz et al., 2004; Kaya et al., 2012].

In the following paragraphs, details of the HPLC methods employed to determine tissue CoQ_{10} status in the laboratories of the authors will be outlined.

Table 1. Gene mutations and associated clinical phenotypes of patients with CoQ_{10} deficiency

Gene	Molecular genetics	Description
PDSS1	10p12.1 ASP308GLU (D308E)	Multisystem disease with early-onset deafness, optic atrophy, mild mental retardation, peripheral neuropathy, obesity, livedo reticularis, and cardiac valvulopathy; Mollet et al. [2007]
PDSS2	6q21 GLN322TER (Q322X); SER382LEU (S382L)	Fatal encephalomyopathy and nephrotic syndrome; Lopez et al. [2006]
COQ2	4q21.23	Early-onset infantile encephalomyopathy, nephropathy; Salviati et al. [2005], Diomedi-Camassei et al. [2007] Fatal infantile multiorgan disease including anemia, pancytopenia, liver failure, and renal insufficiency; Mollet et al. [2007]
COQ4	9q34.11 heterozygous 3.9-Mb deletion	Encephalomyopathic disorder, including poor growth, hypotonia, and delayed psychomotor development with moderate mental retardation and an inability to walk at age; Salviati et al. [2012]
COQ6	14q24.3 GLY255ARG (G255R); ALA353ASP (A353D); TRP447TER (Q447X); 1-bp del, c.1383delG; ARG162TER (R162X); TRP188TER (W188X)	Early-onset nephrotic syndrome with sensorineural deafness; Heeringa et al. [2011]
CABC1 (COQ8/ ADCK3)	1q42.13 GLU551LYS (E551K); ARG213TRP (R213W); GLY272VAL (G272V); GLY272ASP (G272D); 1-bp ins, c.1812insG; IVS11+2 T>C; 22-bp del/3-bp ins; TYR514CYS (Y514C); 3-bp del, 1750ACC 3-bp del, c.1750delAAC; 993C-T c. 993C>T, Ex8 → (Lys314_ Gln360del); GLY549SER (G549S)	Autosomal recessive childhood-onset cerebellar ataxia with cerebellar atrophy, seizures, developmental delay, and hyperlactatemia; Mollet et al. [2008], Lagier-Tourenne et al. [2008]
COQ9	16q13 ARG244TER (R244X)	Infant with severe fatal CoQ_{10} deficiency; Duncan et al. [2009]

HPLC-UV Conditions

Total tissue CoQ_{10} status is quantified by reverse-phase HPLC with UV detection at 275 nm according to the method of Duncan et al. [2005]. CoQ_{10} is separated on a HPLC column (Techsphere ODS 5 μm, 150 × 4.6 mm). The mobile phase consists of ethanol:methanol:60% perchloric acid; 700:300:1.2 to which 7 g of sodium perchlorate are added [Boitier et al., 1998]. The flow rate is maintained at 0.7 ml/min. Ubiquinone species are detected at 275 nm. This HPLC method has been used to determine the CoQ_{10} status of skeletal muscle and blood mononuclear cells which were prepared and extracted according to the method of Duncan et al. [2005].

HPLC-ED Conditions

The total CoQ_{10} concentration is quantified by reverse-phase HPLC with electrochemical detection (Coulochem II, ESA, Mass., USA) according to a previously reported procedure [Montero et al., 2008]. Briefly, CoQ_9 and CoQ_{10} are separated in a nucleosil C-18 column

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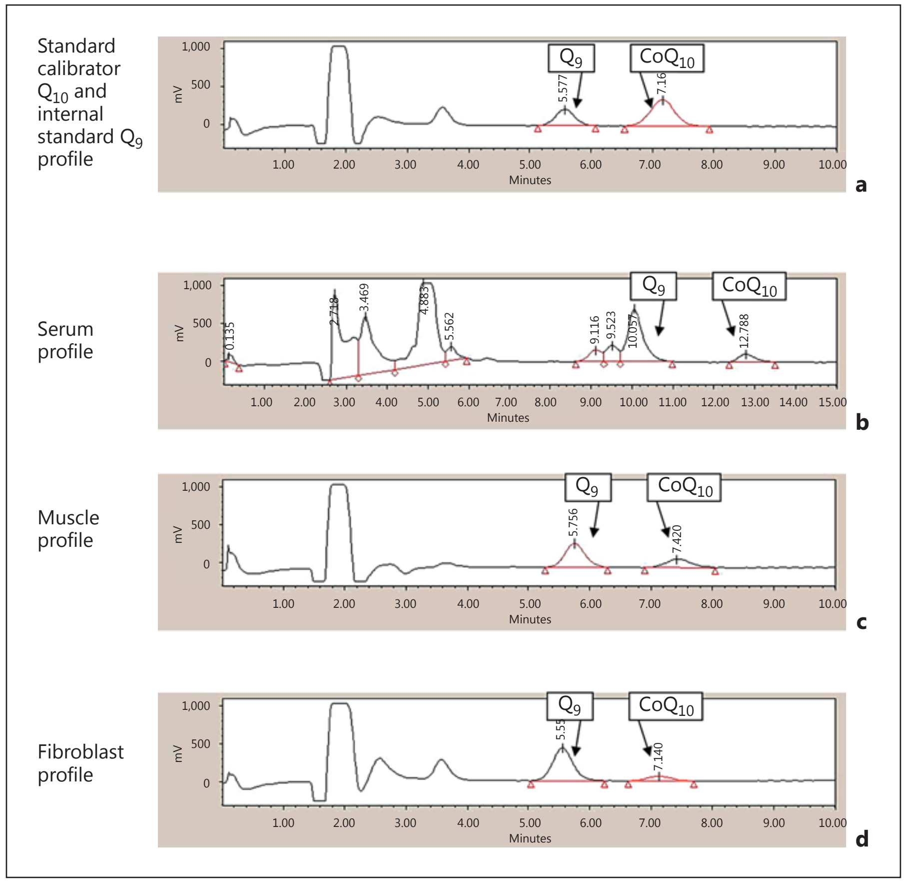

Fig. 2. Reverse-phase HPLC-ED chromatograms of CoQ_9 and CoQ_{10} in **a** standard calibrator Q_{10} and internal standard Q_9 profile, **b** serum profile, **c** muscle profile, and **d** fibroblast profile.

(5 µm, 25 × 0.4 cm, Teknokroma, Barcelona, Spain). The mobile phase consists of 20 mM of lithium perchlorate in ethanol/methanol (40/60). Electrochemical detector cells were set to –600 mV (conditioning cell, Model 5021) and +600 mV (analytical cell, Model 5010).

This HPLC method has been employed to assess the CoQ_{10} status of plasma, skeletal muscle, and fibroblasts (fig. 2) which were prepared and extracted according to the method of Montero et al. [2008].

Internal Standards

A major difficulty encountered when assessing CoQ_{10} status in tissue is the lack of commercially available non-physiological internal standards (IS). In most cases coenzyme Q_9 is the IS of choice [Okamoto et al., 1988]. Unfortunately, as a result of dietary contamination and synthesis by intestinal microorganisms, CoQ_9 has been detected in human tissue and plasma samples contributing up to 2–7% of the total ubiquinone pool [Weber et al., 1997]. To avoid the possible influence of endogenous ubiquinones when evaluating tissue CoQ_{10} status, the non-physiological ubiquinones, di-ethoxy-CoQ_{10} [Edlund, 1988] and di-propoxy-CoQ_{10} [Duncan et al., 2005], have been employed as IS in these determinations.

Tissue Assessment

Plasma, Blood Mononuclear Cells, and Platelet Assessment

Clinical assessment of CoQ_{10} deficiency is generally based on plasma measurements, and the reference interval established for plasma CoQ_{10} status appears to range from 0.5 to 1.7 µM [Molyneux et al., 2008]. Plasma CoQ_{10} levels are also monitored following supplementation therapy to assess absorption and bioavailability of CoQ_{10} formulations. Plasma CoQ_{10} levels as high as 10.7 µM have been reached following supplementation with solubilised formulations of ubiquinol [Bhagavan and Chopra, 2007]. Higher than 'normal' levels of plasma CoQ_{10} appear requisite to facilitate tissue uptake and allow transfer across the blood brain barrier, although these levels may vary for different tissue [Bhagavan and Chopra, 2007]. In Parkinson's disease, plasma CoQ_{10} levels of 4.6 μM were reported by Shults et al. [2002] to be the most efficacious in slowing functional decline in patients. In contrast, a plasma level of 2.8 μM showed the highest therapeutic benefit in the treatment of congestive heart disease patients [Belardinelli et al., 2006]. Gender does not appear to influence plasma CoQ_{10} status [Molyneux et al., 2005]; however, the effect of age upon plasma CoQ_{10} levels is as yet uncertain, with studies reporting both a positive correlation with age and others finding no age effects [Miles et al., 2004; Duncan et al., 2005]. Plasma CoQ_{10} status is influenced by both dietary supply and hepatic biosynthesis [Hargreaves et al., 2005]. This is in contrast to other tissues which are dependent upon de novo biosynthesis [Kalen et al., 1987]. The effect of diet is of particular importance, since CoQ_{10} has a relatively long circulatory half-life (approx. 24 h), and dietary intake may contribute up to 25% of the total amount of plasma CoQ_{10} [Weber et al., 1997].

Plasma CoQ_{10} status is highly dependent upon the concentration of lipoproteins which are the major carriers of CoQ_{10} in the circulation, with approximately 58% of total plasma CoQ_{10} being associated with low-density lipoprotein or LDL fraction [Karlsson et al., 1992; McDonnell and Archbold, 1996]. Therefore, in view of its dependence upon both dietary intake and lipoprotein concentration, plasma CoQ_{10} status may not truly reflect tissue levels [Duncan et al., 2005]. It has been suggested that plasma CoQ_{10} levels should be expressed as a ratio to either total plasma cholesterol or LDL cholesterol in order to be of diagnostic value [Kontush et al., 1997; Tomasetti et al., 1999]. Furthermore, expressing plasma CoQ_{10} as a ratio to total cholesterol appears to exclude any influence of age on this parameter [Wolters and Hahn, 2003; Molyneux et al., 2005].

Assessment of blood mononuclear cells has been suggested as an alternative surrogate to evaluate endogenous CoQ_{10} status [Duncan et al., 2005]. Mononuclear cells are easily isolated from EDTA/Li-Heparin blood, and the CoQ_{10} status of these cells has been reported to correlate with that of skeletal muscle [Duncan et al., 2005]. Blood mononuclear cells are also reported to reflect changes in cellular status following supplementation [Turunen et al., 2004]. This is illustrated by the patient described in the study of Duncan et al. [2005] who was found to have a CoQ_{10} deficiency in blood mononuclear cells (20 pmol/mg of protein: reference interval 37–133 pmol/mg). However, following CoQ_{10} supplementation at 300 mg/day for 2 months, the mononuclear cell CoQ_{10} status of the patient increased to 42 pmol/mg, and this was accompanied by an improvement in mobility; the patient went from being a 'bottom shuffler' to being able to walk upright with the aid of assistance. Platelets have also been used as surrogates to evaluate endogenous CoQ_{10} levels in clinical studies [Shults et al., 1997; Mortensen et al., 1998]. Furthermore, the CoQ_{10} status of platelets was also found to increase following CoQ_{10} supplementation indicating these cell fragments may also be used to monitor the effect of CoQ_{10} supplementation on endogenous levels [Niklowitz et al., 2004].

Skeletal Muscle

Skeletal muscle is generally considered as the tissue of choice for CoQ_{10} assessment, and this tissue has been used in diagnosis of CoQ_{10} deficiency since the first cases of this deficiency were reported by Ogasahara et al. [1989]. However, in view of the importance of this tissue in the diagnosis of CoQ_{10} deficiency, there appears to be no universally accepted units in which to represent skeletal muscle CoQ_{10} status, and therefore it is difficult to compare reference ranges between laboratories (table 2). As is shown in table 2, skeletal muscle CoQ_{10} status can be represented in either units of μg/g fresh weight of tissue or as pmol/mg of protein (nmol/g of protein). Interestingly, although HPLC-UV and HPLC-ED detection methods were used to determine skeletal muscle CoQ_{10} status in the studies reported by Rahman et al. [2001] and Montero et al. [2008], respectively, the reference ranges reported in these studies are markedly similar. In the study by Montero et al. [2008], a patient was described in whom a decreased skeletal muscle CoQ_{10} status was suspected in view of a severe reduction in the activities of the ETC CoQ_{10}-dependent enzymes, complexes II–III (succinate: cytochrome c reductase; EC. 1.3.5.1 + 1.10.2.2) and I–III (NADH: cytochrome c reductase; EC 1.6.5.3 + 1.10.2.2). However, the patient was found to have a normal level of skeletal muscle CoQ_{10} when related to protein (125 nmol/g; reference values: 110–480 nmol/g). When the muscle CoQ_{10} content was related to citrate synthase (CS) activity, the mitochondrial marker enzyme [Selak et al., 2000], evidence of a CoQ_{10} deficiency, was apparent (1.16 nmol/CS units; reference values 2.68–8.47 nmol/CS units). A possible explanation for this observation offered by the authors was the possibility that the high degree of muscle injury the patient was experiencing [rhabdomyolysis and elevated plasma creatine kinase levels (250,000 UI; reference values 50–250 UI)] may have resulted in a depletion of skeletal muscle protein. Therefore, when the CoQ_{10} status was related to total muscle protein content,

Table 2. Reference values for CoQ_{10} levels in muscle and fibroblasts expressed in the different units reported in the literature

Method for analysis	Muscle			Fibroblast	Authors
	CoQ_{10} (nmol/g protein)	CoQ_{10} (nmol/CS units)[a]	CoQ_{10}/gram of tissue	CoQ_{10}	
HPLC-UV (275 nm)	1,440–2,260 (1,811)[b]			43–51 (48) ng/mg of protein	Ogasahara et al. [1989]
HPLC-UV	140–580 (241)			39–75 (62) nmol/g protein	Duncan et al. [2009]
HPLC-ED	117–312 (214)				Miles et al. [2004]
HPLC-ED	110–480 (231)	2.7–8.5 (5.4)		48–112 (67) nmol/g protein	Montero et al. [2008]
HPLC-UV (275 nm)		2.7–7.0 (4.7)			Horvath et al. [2006]
HPLC-UV (275 nm)	140–580 (213)				Rahman et al. [2001]
HPLC-UV (275 nm)			24.0–39.5 (31.5) nmol/g of wet tissue		Sacconi et al. [2010]
HPLC-ED			20–79 (37.4) μmol/g tissue		Terracciano et al. [2012]
HPLC-ED			21.7–88.7 (33.0) μmol/g		Pastore et al. [2012]
HPLC-ED			18.5–45.7 (32.1) μg/g	34–70.4 (52.2) ng/mg	Lopez et al. [2006]
HPLC-ED				50.3–66.7 (58.5) ng/mg protein	Lagier-Tourenne et al. [2008]
HPLC-tandem mass spectrometry			12.6–51.8 (32.2) pmol/mg	56–184 (120) nmol/g protein	Mollet et al. [2008]
HPLC-tandem mass spectrometry				2.0–2.8 (2.4) nmol/CS units	Buján et al. [2013]

Data is presented as range (mean) values. Regarding the reference values in muscle and fibroblasts, most authors report consistent reference intervals, although noticeable differences are present in others. In view of this, the use of validated protocols together with an external quality control program seems necessary to minimize such differences.
[a] CS = Citrate synthase.
[b] These data are reported as ng/mg of muscle mitochondrial protein.

this may result in a 'false-negative' result. Since approximately 50% of cellular CoQ_{10} is present in the mitochondria [Ernster and Dallner, 1995], expressing muscle CoQ_{10} status in relation to CS activity may have important diagnostic value (table 2). This is especially important in mitochondrial myopathies where excessive proliferation of mitochondria has been reported in muscle [DiMauro, 2004] and therefore expressing CoQ_{10} to CS activity which takes into account that the mitochondrial enrichment of the sample may highlight evidence of a deficiency which may not be identifiable if CoQ_{10} status is solely related to total protein.

Decreased activity of either ETC complex I–III and/or complex II–III is also indicative of a CoQ_{10} deficiency as the activity of these linked enzymes is dependent upon endogenous CoQ_{10} [Rahman et al., 2001]. Furthermore, the study by Montero et al. [2008] has suggested that complex II–III activity may be a more sensitive marker of a diminution in CoQ_{10} status than that of complex I–III. However, normal levels of complex I–III or II–III activity do not exclude a decrease in muscle CoQ_{10} status as has previously been observed in patients with the ataxic phenotype of CoQ_{10} deficiency [Lamperti et al., 2003]. In view of the essential role ubiquinol plays in pyrimidine synthesis [Lopez et al., 2006], mitochondrial DNA depletion syndrome may also be associated with a decrease in CoQ_{10} status as has been reported by Montero et al. [2009]. Therefore, the assessment of muscle CoQ_{10} status in patients who present with multiple ETC deficiencies should not be discouraged. Furthermore, in view of the association between mitochondrial DNA mutations and muscle coenzyme Q_{10} deficiency, assessment of muscle

CoQ_{10} status should be considered in addition to the determination of ETC enzyme activities in patients with suspected mitochondrial disease [Sacconi et al., 2010]. Decreased glycerol 3-phosphate dehydrogenase and/or dihydroorotate cytochrome c reductase activity may also indicate evidence of decreased muscle CoQ_{10} levels as these enzymes have been reported to be especially sensitive to perturbations in CoQ_{10} status [Rotig et al., 2000].

Fibroblasts

Assessment of fibroblast CoQ_{10} status should also be considered in the diagnosis of CoQ_{10} deficiency. Published reference ranges for fibroblast CoQ_{10} status are shown in table 2. In view of the suggested tissue specificity of CoQ_{10} deficiency, a normal level of CoQ_{10} in fibroblasts does not exclude a deficit in CoQ_{10} status in other tissues [Ogasahara et al., 1989]. Indeed, normal levels of fibroblast CoQ_{10} have been reported in patients with genetically confirmed defects in CoQ_{10} biosynthesis [Lagier-Tourenne et al., 2008]. In contrast, however, fibroblast assessment has been used to reveal evidence of a CoQ_{10} deficiency in a patient with a normal muscle CoQ_{10} status [Montero et al., 2008]. Fibroblasts also provide a means of assaying CoQ_{10} biosynthesis by studying the incorporation of ^{14}C-p-hydroxybenzoate, ^{3}H-mevalonate, and/or ^{3}H-decaprenyl-pyrophosphate into CoQ_{10} [Lopez et al., 2006; Quinzii et al., 2006]. These radiolabelled incorporation studies can be used to confirm a deficiency in CoQ_{10} biosynthesis, identify the position of the defect in the biosynthetic pathway in some cases, as well as to discriminate between primary and secondary CoQ_{10} deficiencies.

Cerebral Spinal Fluid

In view of the preponderance of neurological dysfunction associated with CoQ_{10} deficiency [Mancuso et al., 2010], the ability to assess cerebral CoQ_{10} status would be of considerable diagnostic value. Cerebral spinal fluid (CSF) is considered the appropriate surrogate to assess cerebral CoQ_{10} status. However, in view of the low levels of CoQ_{10} detected in CSF with HPLC-UV, detection would be insufficiently sensitive for this analysis [Duncan et al., 2005]. Tentative reference ranges for CSF CoQ_{10} levels of 1.18–4.91 nM established from a patient cohort aged 9–18 years, n = 15 [Artuch et al., 2004] and 5.7–9 nM established from a patient cohort aged 0.1–22 years, n = 17 [Duberley et al., 2012], respectively, have been reported. The discrepancies in these ranges may in part result from the different analytical techniques and sample preparations employed for this determination. In the study by Artuch et al. [2004], CSF samples were filtered by passing through a 10,000-NMWL column prior to analysis by HPLC-EC detection. In contrast, tandem spectrometry was employed to determine the CoQ_{10} status in unfiltered CSF in the study by Duberley et al. [2012]. A further factor, which may also have contributed to this disparity, is the different ages of the 'disease control' patients used to establish these reference ranges. Although Isobe et al. [2010] reported no correlation between age and CSF CoQ_{10} status, this study was undertaken solely in adults aged 65.8 ± 12.4 years (mean ± SD), and CSF was not investigated from children. Therefore, in order to establish a more reliable and robust reference interval for CSF CoQ_{10} status, further studies are required that evaluate the effects of age, gender, as well as the rostral-caudal gradient upon CSF levels of this ubiquinone.

Discussion/Conclusion

The actual prevalence of human CoQ_{10} deficiency is at present unknown, but it is suspected that this condition is under-diagnosed [Rahman et al., 2012]. This is compounded by the lack of specialist centres which are able to determine tissue CoQ_{10} status together with the extreme clinical heterogeneity of this condition [Rahman et al., 2012]. It is recommended that the CoQ_{10} status is determined in the muscle biopsies of all patients with suspected mitochondrial disease. Once evidence of a CoQ_{10} deficiency is detected, further studies will be required to elucidate the underlying cause of this defect. Genetic investigations and radiolabelled biosynthetic studies in fibroblasts may help to distinguish between primary or secondary causes of the deficiency. However, in a number of patients with CoQ_{10} deficiency it has not been possible to elucidate the underlying cause of the defect [Rahman et al., 2012].

Since muscle biopsies may not always be available, there is a need for a less invasive means to assess tissue CoQ_{10} status. Although there are some concerns over the diagnostic value of plasma CoQ_{10} levels, platelet and blood mononuclear cell determinations may offer an alternative means for this assessment.

It has been suggested that there may be tissue specific isoenzymes in the CoQ_{10} biosynthetic pathway; therefore the CoQ_{10} status of one tissue may not reflect that of another [Ogasahara et al., 1989]. Since neurological dysfunction is a constant clinical feature in CoQ_{10} deficiency syndromes, although some defects may be expressed in muscle or peripheral tissue, other defects (such as those

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in cerebral CoQ_{10} biosynthesis) may not be expressed and may remain undiagnosed. The ability to accurately assess CSF CoQ_{10} status may therefore enhance the diagnosis yield of patients with neurological dysfunction and previously undiagnosed cerebral CoQ_{10} deficiency. In view of the differences in the tissue of choice for CoQ_{10} assessment, units in which CoQ_{10} is expressed and the reference intervals used for this diagnosis between laboratories a more unified approach is required for monitoring patients and their treatment. The establishment of an external quality control (Ex-QC) scheme for the measurement of tissue CoQ_{10} status is suggested for laboratories offering this clinical diagnostic service. At present, a trial Ex-QC scheme is running between laboratories in the UK and Spain and, if successful, will be offered on a more global scale.

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