Biochemical Diagnosis of Coenzyme Q₁₀ Deficiency

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Abstract

Coenzyme Q₁₀ (CoQ₁₀) 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 CoQ₁₀ deficiency which relies upon biochemical assessment of tissue CoQ₁₀ status. In this article, we review the biochemical methods used in the diagnosis of human CoQ₁₀ deficiency and indicate the most appropriate tissues for this evaluation.

Coenzyme Q₁₀ (CoQ₁₀) 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 CoQ₁₀, 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, CoQ₁₀ 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 CoQ₁₀ status could conceivably contribute to disease pathophysiology by causing a failure in energy metabolism and compromising cellular antioxidant status.

The first cases of CoQ₁₀ 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 CoQ₁₀ deficiency appears to have a particularly heterogeneous clinical presentation. However, there appear to be 5 distinct clinical phenotypes: encephalomyopathy, severe infantile multisystemic disease, nephropa-
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<sub>10</sub> biosynthetic pathway, have been associated with human CoQ<sub>10</sub> deficiency (table 1). However, in the preponderance of patients with CoQ<sub>10</sub> deficiency, it has not been possible to identify the underlying genetic cause [Rahman et al., 2012]. The genetic diagnosis of CoQ<sub>10</sub> deficiency is complicated by the fact that the CoQ<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> deficiency and to indicate the most appropriate tissues for this evaluation.

**Methods Used to Determine Tissue CoQ<sub>10</sub> Status**

The most common laboratory procedures used for diagnosis of CoQ<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> concomitantly by HPLC-ED analysis, diagnostically, the determination of total tissue CoQ<sub>10</sub> status is sufficiently accurate to detect human CoQ<sub>10</sub> deficiencies. Simultaneous measurement of both reduced and oxidized forms of CoQ<sub>10</sub> usually requires a complex pre-analytical management of samples, and chromatographically, this is more complicated than the measurement of total CoQ<sub>10</sub>. Furthermore, the propensity of ubiquinol to oxidise to CoQ<sub>10</sub> 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<sub>10</sub> is probably more suitable for research purposes rather than for clinical diagnosis. However, in appropriately handled tissue samples the ratio of ubiquinol:CoQ<sub>10</sub> 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<sub>10</sub> status in the laboratories of the authors will be outlined.
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 (Coulotech 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.

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

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<thead>
<tr>
<th>Gene</th>
<th>Molecular genetics</th>
<th>Description</th>
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<tr>
<td>PDSS1</td>
<td>10p12.1 ASP308GLU (D308E)</td>
<td>Multisystem disease with early-onset deafness, optic atrophy, mild mental retardation, peripheral neuropathy, obesity, livedo reticularis, and cardiac valvulopathy; Mollet et al. [2007]</td>
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<tr>
<td>PDSS2</td>
<td>6q21 GLN322TER (Q322X); SER382LEU (S382L)</td>
<td>Fatal encephalomyopathy and nephrotic syndrome; Lopez et al. [2006]</td>
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<tr>
<td>COQ2</td>
<td>4q21.23</td>
<td>Early-onset infantile encephalomyopathy, nephropathy; Salviati et al. [2005], Diomedi-Camassei et al. [2007]</td>
</tr>
<tr>
<td>COQ4</td>
<td>9q34.11 heterozygous 3.9-Mb deletion</td>
<td>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]</td>
</tr>
<tr>
<td>COQ6</td>
<td>14q24.3 GLY255ARG (G255R); ALA353ASP (A353D); TRP447TER (Q447X); 1-bp del, c.1383delG; ARG162TER (R162X); TRP188TER (W188X)</td>
<td>Early-onset nephrotic syndrome with sensorineural deafness; Heeringa et al. [2011]</td>
</tr>
<tr>
<td>CABC1</td>
<td>1q42.13 GLU551LYS (E551K); ARG213TRP (R213W); GLY272VAL (G272V); GLY272ASP (G272D); 1-bp ins, c.1812insG; IVS11 + 2 T&gt;C; 22-bp del/3-bp ins; TYR514CYS (Y514C); 3-bp del, 1750ACC 3-bp del, c.1750delAAAC; 993C&gt;T c. 993C&gt;T, Ex8 → (Lys314_Gln360del); GLY549SER (G549S)</td>
<td>Autosomal recessive childhood-onset cerebellar ataxia with cerebellar atrophy, seizures, developmental delay, and hyperlactatemia; Mollet et al. [2008], Lagier-Tourenne et al. [2008]</td>
</tr>
<tr>
<td>COQ9</td>
<td>16q13 ARG244TER (R244X)</td>
<td>Infant with severe fatal CoQ_{10} deficiency; Duncan et al. [2009]</td>
</tr>
</tbody>
</table>
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\textsubscript{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\textsubscript{10} status in tissue is the lack of commercially available non-physiological internal standards (IS). In most cases coenzyme Q\textsubscript{9} is the IS of choice [Okamoto et al., 1988]. Unfortunately, as a result of dietary contamination and synthesis by intestinal microorganisms, CoQ\textsubscript{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\textsubscript{10} status, the non-physiological ubiquinones, di-ethoxy-CoQ\textsubscript{10} [Edlund, 1988] and di-propoxy-CoQ\textsubscript{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\textsubscript{10} deficiency is generally based on plasma measurements, and the reference interval established for plasma CoQ\textsubscript{10} status appears to range from 0.5 to 1.7 μM [Molyneux et al., 2008]. Plasma CoQ\textsubscript{10} levels are also monitored following supplementation therapy to assess absorption and bioavailability of CoQ\textsubscript{10} formulations. Plasma CoQ\textsubscript{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\textsubscript{10} appear requisite to facilitate tissue uptake and allow transfer across the blood brain barrier, although these levels may

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**Fig. 2.** Reverse-phase HPLC-ED chromatograms of CoQ\textsubscript{9} and CoQ\textsubscript{10} in a standard calibrator Q\textsubscript{10} and internal standard Q\textsubscript{9} profile, b serum profile, c muscle profile, and d fibroblast profile.
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 Ogashahara 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\(_{6}\)-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,
this may result in a ‘false-negative’ result. Since approximately 50% of cellular CoQ₁₀ is present in the mitochondria [Ernster and Dallner, 1995], expressing muscle CoQ₁₀ 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 [Di-Mauro, 2004] and therefore expressing CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ deficiency as the activity of these linked enzymes is dependent upon endogenous CoQ₁₀ [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 CoQ₁₀ 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 CoQ₁₀ status as has previously been observed in patients with the ataxic phenotype of CoQ₁₀ 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 CoQ₁₀ status as has been reported by Montero et al. [2009]. Therefore, the assessment of muscle CoQ₁₀ 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 Q₁₀ deficiency, assessment of muscle

<table>
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<tr>
<th>Method for analysis</th>
<th>Muscle CoQ₁₀ (nmol/g protein)</th>
<th>Muscle CoQ₁₀ (nmol/CS units)</th>
<th>Muscle CoQ₁₀/gram of tissue</th>
<th>Fibroblast CoQ₁₀ (ng/mg of protein)</th>
<th>Authors</th>
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</thead>
<tbody>
<tr>
<td>HPLC-UV (275 nm)</td>
<td>1,440–2,260 (1,811)</td>
<td></td>
<td></td>
<td>43–51 (48)</td>
<td>Ogasahara et al. [1989]</td>
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<tr>
<td>HPLC-UV</td>
<td>140–580 (241)</td>
<td></td>
<td></td>
<td>39–75 (62)</td>
<td>Duncan et al. [2009]</td>
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<tr>
<td>HPLC-ED</td>
<td>117–312 (214)</td>
<td>2.7–8.5 (5.4)</td>
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<td>48–112 (67)</td>
<td>Miles et al. [2004]</td>
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<tr>
<td>HPLC-UV (275 nm)</td>
<td>110–480 (231)</td>
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<td>Montero et al. [2008]</td>
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<tr>
<td>HPLC-UV (275 nm)</td>
<td>140–580 (213)</td>
<td>2.7–7.0 (4.7)</td>
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<td>Horvath et al. [2006]</td>
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<tr>
<td>HPLC-ED</td>
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<td>24.0–39.5 (31.5)</td>
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<td>Rahman et al. [2001]</td>
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<td></td>
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<td>nmol/g of wet tissue</td>
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<td>Sacconi et al. [2010]</td>
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<td>20–79 (37.4)</td>
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<td>µmol/g tissue</td>
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<td>21.7–88.7 (33.0)</td>
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<td>µmol/g</td>
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<td>18.5–45.7 (32.1)</td>
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<td>µg/g</td>
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<td>34–70.4 (52.2)</td>
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<td>ng/mg</td>
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<td>50.3–66.7 (58.5)</td>
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<td>nmol/mg</td>
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<td>56–184 (120)</td>
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<td>nmol/g protein</td>
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<td></td>
<td>2.0–2.8 (2.4)</td>
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<td></td>
<td></td>
<td></td>
<td>nmol/CS units</td>
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<td>HPLC-tandem mass spectrometry</td>
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<tr>
<td>HPLC-tandem mass spectrometry</td>
<td>12.6–51.8 (32.2)</td>
<td>2.0–2.8 (2.4)</td>
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</table>

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.
CoQ<sub>10</sub> 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<sub>10</sub> levels as these enzymes have been reported to be especially sensitive to perturbations in CoQ<sub>10</sub> status [Rotig et al., 2000].

**Fibroblasts**

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

**Cerebral Spinal Fluid**

In view of the preponderance of neurological dysfunction associated with CoQ<sub>10</sub> deficiency [Mancuso et al., 2010], the ability to assess cerebral CoQ<sub>10</sub> status would be of considerable diagnostic value. Cerebral spinal fluid (CSF) is considered the appropriate surrogate to assess cerebral CoQ<sub>10</sub> status. However, in view of the low levels of CoQ<sub>10</sub> detected in CSF with HPLC-UV, detection would be insufficiently sensitive for this analysis [Duncan et al., 2005]. Tentative reference ranges for CSF CoQ<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> status together with the extreme clinical heterogeneity of this condition [Rahman et al., 2012]. It is recommended that the CoQ<sub>10</sub> status is determined in the muscle biopsies of all patients with suspected mitochondrial disease. Once evidence of a CoQ<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> status. Although there are some concerns over the diagnostic value of plasma CoQ<sub>10</sub> 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<sub>10</sub> biosynthetic pathway; therefore the CoQ<sub>10</sub> status of one tissue may not reflect that of another [Ogasahara et al., 1989]. Since neurological dysfunction is a constant clinical feature in CoQ<sub>10</sub> deficiency syndromes, although some defects may be expressed in muscle or peripheral tissue, other defects (such as those...
in cerebral CoQ10 biosynthesis) may not be expressed and may remain undiagnosed. The ability to accurately assess CSF CoQ10 status may therefore enhance the diagnosis yield of patients with neurological dysfunction and previously undiagnosed cerebral CoQ10 deficiency. In view of the differences in the tissue of choice for CoQ10 assessment, units in which CoQ10 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 CoQ10 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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