Biochemical Diagnosis of Coenzyme Q₁₀ Deficiency

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Key Words

Antioxidant · Cholesterol · Coenzyme Q₁₀ · Disease · Mitochondria · Muscle · Plasma · Tissue

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.

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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 trans-

ferring 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-

Fig. 1. Structure of mitochondrial electron transport chain, showing the electron carrier function of coenzyme Q₁₀ (Q). Red circles indicate proteins with mutations causing CoQ₁₀ 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 CoQ₁₀ biosynthetic pathway, have been associated with human CoQ₁₀ deficiency (table 1). However, in the preponderance of patients with CoQ₁₀ deficiency, it has not been possible to identify the underlying genetic cause [Rahman et al., 2012]. The genetic diagnosis of CoQ₁₀ deficiency is complicated by the fact that the CoQ₁₀ 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 CoQ₁₀ synthesis [Emmanuele et al., 2012].

The purpose of this article is to critically review the current biochemical methods used in the diagnosis of human CoQ₁₀ deficiency and to indicate the most appropriate tissues for this evaluation.

Methods Used to Determine Tissue CoQ₁₀ Status

The most common laboratory procedures used for diagnosis of CoQ₁₀ 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 CoQ₁₀ assessment; however, this method will not be discussed further in this article and the reader is referred to the review by Barshop and Gangoiiti [2007] which discusses this analytical procedure. Although it is possible to determine the reduced (ubiquinol) and oxidized forms of CoQ₁₀ concomitantly by HPLC-ED analysis, diagnostically, the determination of total tissue CoQ₁₀ status is sufficiently accurate to detect human CoQ₁₀ deficiencies. Simultaneous measurement of both reduced and oxidized forms of CoQ₁₀ usually requires a complex pre-analytical management of samples, and chromatographically, this is more complicated than the measurement of total CoQ₁₀. Furthermore, the propensity of ubiquinol to oxidise to CoQ₁₀ 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 CoQ₁₀ is probably more suitable for research purposes rather than for clinical diagnosis. However, in appropriately handled tissue samples the ratio of ubiquinol:CoQ₁₀ 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 CoQ₁₀ status in the laboratories of the authors will be outlined.

Table 1. Gene mutations and associated clinical phenotypes of patients with CoQ₁₀ deficiency

Gene	Molecular genetics	Description
<i>PDSS1</i>	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]
<i>PDSS2</i>	6q21 GLN322TER (Q322X); SER382LEU (S382L)	Fatal encephalomyopathy and nephrotic syndrome; Lopez et al. [2006]
<i>COQ2</i>	4q21.23	Early-onset infantile encephalomyopathy, nephropathy; Salviati et al. [2005], Diomedi-Camassei et al. [2007]
<i>COQ4</i>	9q34.11 heterozygous 3.9-Mb deletion	Fatal infantile multiorgan disease including anemia, pancytopenia, liver failure, and renal insufficiency; Mollet et al. [2007]
<i>COQ6</i>	14q24.3 GLY255ARG (G255R); ALA353ASP (A353D); TRP447TER (Q447X); 1-bp del, c.1383delG; ARG162TER (R162X); TRP188TER (W188X)	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]
<i>CABC1</i> (<i>COQ8/</i> <i>ADCK3</i>)	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)	Early-onset nephrotic syndrome with sensorineural deafness; Heeringa et al. [2011]
<i>COQ9</i>	16q13 ARG244TER (R244X)	Autosomal recessive childhood-onset cerebellar ataxia with cerebellar atrophy, seizures, developmental delay, and hyperlactatemia; Mollet et al. [2008], Lagier-Tourenne et al. [2008]
		Infant with severe fatal CoQ ₁₀ deficiency; Duncan et al. [2009]

HPLC-UV Conditions

Total tissue CoQ₁₀ status is quantified by reverse-phase HPLC with UV detection at 275 nm according to the method of Duncan et al. [2005]. CoQ₁₀ 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 deter-

mine the CoQ₁₀ 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 CoQ₁₀ 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, CoQ₉ and CoQ₁₀ are separated in a nucleosil C-18 column

Fig. 2. Reverse-phase HPLC-ED chromatograms of CoQ₉ and CoQ₁₀ in **a** standard calibrator Q₁₀ and internal standard Q₉ profile, **b** serum profile, **c** muscle profile, and **d** fibroblast profile.

(5 μ m, 25 $\times$ 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 CoQ₁₀ 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 CoQ₁₀ status in tissue is the lack of commercially available non-physiological internal standards (IS). In most cases coenzyme Q₉ is the IS of choice [Okamoto et al., 1988]. Unfortunately, as a result of dietary contamination and synthesis by intestinal microorganisms, CoQ₉ 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 CoQ₁₀ status, the non-

physiological ubiquinones, di-ethoxy-CoQ₁₀ [Edlund, 1988] and di-propoxy-CoQ₁₀ [Duncan et al., 2005], have been employed as IS in these determinations.

Tissue Assessment

Plasma, Blood Mononuclear Cells, and Platelet Assessment

Clinical assessment of CoQ₁₀ deficiency is generally based on plasma measurements, and the reference interval established for plasma CoQ₁₀ status appears to range from 0.5 to 1.7 μ M [Molyneux et al., 2008]. Plasma CoQ₁₀ levels are also monitored following supplementation therapy to assess absorption and bioavailability of CoQ₁₀ formulations. Plasma CoQ₁₀ levels as high as 10.7 μ M have been reached following supplementation with solubilised formulations of ubiquinol [Bhagavan and Chopra, 2007]. Higher than 'normal' levels of plasma CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ status [Molyneux et al., 2005]; however, the effect of age upon plasma CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ has a relatively long circulatory half-life (approx. 24 h), and dietary intake may contribute up to 25% of the total amount of plasma CoQ₁₀ [Weber et al., 1997].

Plasma CoQ₁₀ status is highly dependent upon the concentration of lipoproteins which are the major carriers of CoQ₁₀ in the circulation, with approximately 58% of total plasma CoQ₁₀ 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 CoQ₁₀ status may not truly reflect tissue levels [Duncan et al., 2005]. It has been suggested that plasma CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ status [Duncan et al., 2005]. Mononuclear cells are easily isolated from EDTA/Li-Heparin blood, and the CoQ₁₀ 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 CoQ₁₀ deficiency in blood mononuclear cells (20 pmol/mg of protein; reference interval 37–133 pmol/mg). However, following CoQ₁₀ supplementation at 300 mg/day for 2 months, the mononuclear cell CoQ₁₀ status of the pa-

tient 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 CoQ₁₀ levels in clinical studies [Shults et al., 1997; Mortensen et al., 1998]. Furthermore, the CoQ₁₀ status of platelets was also found to increase following CoQ₁₀ supplementation indicating these cell fragments may also be used to monitor the effect of CoQ₁₀ supplementation on endogenous levels [Niklovitz et al., 2004].

Skeletal Muscle

Skeletal muscle is generally considered as the tissue of choice for CoQ₁₀ assessment, and this tissue has been used in diagnosis of CoQ₁₀ 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 CoQ₁₀ deficiency, there appears to be no universally accepted units in which to represent skeletal muscle CoQ₁₀ status, and therefore it is difficult to compare reference ranges between laboratories (table 2). As is shown in table 2, skeletal muscle CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ status was suspected in view of a severe reduction in the activities of the ETC CoQ₁₀-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 CoQ₁₀ when related to protein (125 nmol/g; reference values: 110–480 nmol/g). When the muscle CoQ₁₀ content was related to citrate synthase (CS) activity, the mitochondrial marker enzyme [Selak et al., 2000], evidence of a CoQ₁₀ 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 CoQ₁₀ status was related to total muscle protein content,

Table 2. Reference values for CoQ₁₀ 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)		24.0–39.5 (31.5) nmol/g of wet tissue		Rahman et al. [2001]
HPLC-UV (275 nm)			20–79 (37.4) μmol/g tissue		Sacconi et al. [2010]
HPLC-ED			21.7–88.7 (33.0) μmol/g		Terracciano et al. [2012]
HPLC-ED			18.5–45.7 (32.1) μg/g	34–70.4 (52.2) ng/mg	Pastore et al. [2012]
HPLC-ED				50.3–66.7 (58.5) ng/mg protein	Lopez et al. [2006]
HPLC-ED				56–184 (120) nmol/g protein	Lagier-Tourenne et al. [2008]
HPLC-tandem mass spectrometry			12.6–51.8 (32.2) pmol/mg	2.0–2.8 (2.4) nmol/CS units	Mollet et al. [2008]
HPLC-tandem mass spectrometry					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 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 [DiMauro, 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

CoQ₁₀ 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 CoQ₁₀ levels as these enzymes have been reported to be especially sensitive to perturbations in CoQ₁₀ status [Rotig et al., 2000].

Fibroblasts

Assessment of fibroblast CoQ₁₀ status should also be considered in the diagnosis of CoQ₁₀ deficiency. Published reference ranges for fibroblast CoQ₁₀ status are shown in table 2. In view of the suggested tissue specificity of CoQ₁₀ deficiency, a normal level of CoQ₁₀ in fibroblasts does not exclude a deficit in CoQ₁₀ status in other tissues [Ogasahara et al., 1989]. Indeed, normal levels of fibroblast CoQ₁₀ have been reported in patients with genetically confirmed defects in CoQ₁₀ biosynthesis [Lagier-Tourenne et al., 2008]. In contrast, however, fibroblast assessment has been used to reveal evidence of a CoQ₁₀ deficiency in a patient with a normal muscle CoQ₁₀ status [Montero et al., 2008]. Fibroblasts also provide a means of assaying CoQ₁₀ biosynthesis by studying the incorporation of ¹⁴C-p-hydroxybenzoate, ³H-mevalonate, and/or ³H-decaprenyl-pyrophosphate into CoQ₁₀ [Lopez et al., 2006; Quinzii et al., 2006]. These radiolabelled incorporation studies can be used to confirm a deficiency in CoQ₁₀ biosynthesis, identify the position of the defect in the biosynthetic pathway in some cases, as well as to discriminate between primary and secondary CoQ₁₀ deficiencies.

Cerebral Spinal Fluid

In view of the preponderance of neurological dysfunction associated with CoQ₁₀ deficiency [Mancuso et al., 2010], the ability to assess cerebral CoQ₁₀ status would be of considerable diagnostic value. Cerebral spinal fluid (CSF) is considered the appropriate surrogate to assess cerebral CoQ₁₀ status. However, in view of the low levels of CoQ₁₀ detected in CSF with HPLC-UV, detection would be insufficiently sensitive for this analysis [Duncan et al., 2005]. Tentative reference ranges for CSF CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ status together with the extreme clinical heterogeneity of this condition [Rahman et al., 2012]. It is recommended that the CoQ₁₀ status is determined in the muscle biopsies of all patients with suspected mitochondrial disease. Once evidence of a CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ status. Although there are some concerns over the diagnostic value of plasma CoQ₁₀ 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 CoQ₁₀ biosynthetic pathway; therefore the CoQ₁₀ status of one tissue may not reflect that of another [Ogasahara et al., 1989]. Since neurological dysfunction is a constant clinical feature in CoQ₁₀ 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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