Short Communication

The diagnosis of carnitine palmitoyltransferase II deficiency is now possible in small skeletal muscle biopsies

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Carnitine palmitoyltransferase II (CPT II) converts acylcarnitine esters to the corresponding acyl-CoA derivatives within mitochondria prior to β-oxidation. CPT II deficiency (McKusick 255110) may present early in life, with patients displaying hypoketonaemia, hypoglycaemia, cardiomyopathy or multiorgan failure (Hug et al 1994). A neonatal case with a lethal myopathy has also been described (Land et al 1995). However, the most common presentation of CPT II deficiency is in adulthood, invariably characterized by exercise-induced rhabdomyolysis and myoglobinuria, especially in the fasted state. In our laboratory we routinely measure mitochondrial respiratory chain enzyme (MRCE) activities in small skeletal muscle biopsies (50–100mg). However, once the diagnosis of MRCE deficiency has been excluded, the ability to measure CPT activity in the muscle biopsies of patients suspected of having MRCE/β-oxidation defects would improve the differential diagnosis. We describe the contribution made by enzymatic measurement in skeletal muscle homogenates to the diagnosis of CPT II deficiency.

PATIENT AND METHODS

The patient was a 42-year-old male who presented with exercise-induced muscle pain and stiffness. These symptoms were exacerbated by fasting and characterized by recurrent episodes of rhabdomyolysis causing acute renal failure that needed dialysis.

Skeletal muscle biopsy samples (50–100mg) were homogenized by the method of Heales and colleagues (1996) and the homogenates were subjected to three cycles of
freezing/thawing. The homogenates were then assayed for CPT activity by measuring the formation of palmitoyl-[methyl-14C]carnitine from 1-[methyl-14C]carnitine and palmitoyl-CoA with or without the presence of malonyl-CoA (100 μmol/L); in the presence of malonyl-CoA, only CPT II activity is recorded (Demaugre et al 1991). Citrate synthase activity was measured by the reaction of free CoA with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) (Shepherd and Garland 1969). All enzymatic activities were assayed at 30°C. Protein concentrations were measured by the Lowry method, using bovine serum albumin (BSA) as standard.

A reference interval was established from 11 ‘disease controls’ who had no detectable mitochondrial electron transport chain deficiency and showed no evidence of a β-oxidation defect.

RESULTS AND DISCUSSION

The CPT activity measured by the radiometric assay was found to be linear with time over an incubation period of 20 min, and was proportional to protein over the range 0.2–1.4 mg. Malonyl-CoA inhibition of total CPT activity was found to be approximately 55%; this was in agreement with previous studies in skeletal muscle mitochondria (Saggerson and Carpenter 1981). CPT activity was then measured in 11 ‘disease controls’ to establish a reference interval. CPT activities were expressed both as nmol/min per mg protein and as ratio to the citrate synthase activity (CS), to compensate for mitochondrial enrichment of the muscle homogenate (Heales et al 1996). Although total CPT (I + II) and CPT I activities of the patient’s muscle homogenate were within the normal range, CPT II activity was markedly reduced, being 16.1% of the lower reference interval (nmol/min per mg) and 10% of the lower reference interval when expressed as a ratio to CS (Table 1). The reduced enzymatic activity in the muscle homogenate suggested that the patient had a marked CPT II deficiency.

Further evidence for this diagnosis was obtained from oxidation studies in cultured fibroblasts; [9,10-3H]myristate, [9,10-3H]palmitate and [9,10-3H]oleate gave activities of 71%, 53% and 46%, respectively, of simultaneous controls. In addition, fibroblast CPT II activity was 9.0% of the control level (Dr R. J. A. Wanders, Laboratory for Genetic Metabolic Diseases, University of Amsterdam, The Netherlands).

This study illustrates that it is now possible to measure, CPT I + II, CPT II and, consequently, CPT I in frozen skeletal muscle biopsies in addition to the measure-

| Table 1 CPT: CS ratios for a CPT II-deficient patient and control skeletal muscle homogenates |
|-------------------------------------------------|-----------------|-----------------|
| Patient                                         | Reference intervala |
| CPT (I + II)/CS                                 | 0.0051           | 0.0030–0.0170   |
| CPT I/CS                                        | 0.0049           | 0.0020–0.0100   |
| CPT II/CS                                       | 0.0002           | 0.0020–0.0080   |

a Reference interval was established from mean ± 2 SD, n = 11 ‘disease controls’.