Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy

Jochen Schaefer, Sandra Jackson, Franco Taroni, Peter Swift, Douglass M Turnbull

Abstract

Objectives—Carnitine palmitoyltransferase (CPT) deficiency is one of the most common defects of mitochondrial fatty acid oxidation. Two different enzymes (CPT-I and CPT-II) are involved. Due to problems in measuring enzyme activity, relatively little is known about the substrate specificity of each of the human enzymes. This is of considerable importance in the treatment of patients. The objectives were to establish a reliable method for the measurement of CPT activity in whole cells, to characterise the substrate specificity of each enzyme, and finally, to determine if medium chain triglycerides would be of benefit in the treatment of deficient patients.

Methods—A simple permeabilisation technique was used which allows the measurement of CPT activity in a small amount of cultured skin fibroblasts or peripheral blood cells. Using this technique three patients were identified with CPT deficiency. In two of these patients, one with CPT-I deficiency and one with CPT-II deficiency, a complete substrate specificity profile of the mitochondrial carnitine acyltransferases was established for all saturated even chain acyl-CoA esters.

Results—For both enzymes the highest CPT activity was with C12-CoA. About 70% of total cellular carnitine octanoyltransferase activity was due to mitochondrial CPT. As CPT is involved in the transport of medium chain fatty acids the metabolic response of a patient with CPT-II deficiency to dietary medium chain triglycerides was assessed. Despite the normal production of ketone bodies there was a significant medium chain dicarboxylic aciduria in the patient, indicating a limited capacity of the CPT independent mitochondrial uptake of medium chain fatty acids.

Conclusions—CPT deficiency can easily be diagnosed in permeabilised cultured skin fibroblasts. Both CPT-I and CPT-II are more active with medium chain length substrates than previously assumed. Care should therefore be taken in the treatment of these patients with medium chain triglycerides.

Keywords: fatty acid oxidation defects; carnitine palmitoyltransferases; fibroblast permeabilisation; medium chain triglyceride therapy

The mitochondrial β-oxidation of long chain fatty acids is a major source of energy production in the cell, particularly in heart, liver, and skeletal muscle. In skeletal muscle fatty acids supply about 70% of the energy required during prolonged exercise and defects of fatty acid oxidation are an important cause of muscle disease.

Mitochondrial β-oxidation is a complex process which requires the concerted action of several enzymes. The carnitine palmitoyltransferase (CPT) enzyme system catalyses the transport of long chain fatty acids into the mitochondria and represents the first obligatory step in the oxidation of fatty acids. It consists of two immunologically distinct membrane bound enzymes CPT-I and CPT-II. CPT-I is associated with the outer mitochondrial membrane, is specifically inhibited by malonyl-CoA, and exists in at least two different tissue specific isoforms. By contrast, CPT-II is located in the inner mitochondrial membrane, is insensitive to malonyl-CoA, and is conserved across tissue line. The enzymes are products of two separate gene loci which have recently been cloned and sequenced. In addition to the mitochondrial enzymes, other carnitine acyltransferases have been identified in peroxisomes and microsomes.

Inherited deficiencies of CPT-I and CPT-II have been described. Deficiency of CPT-I is a rare disorder and usually presents in infancy with recurrent episodes of hypoketotic hypoglycaemia, which are often triggered by fasting and accompanied by a decreased level of consciousness and hepatomegaly. Skeletal muscle dysfunction has not been reported in this disorder. By contrast, deficiency of CPT-II occurs in two distinct phenotypes: in infants CPT-II deficiency may be a generalised lethal disease which is characterised by hypoketotic hypoglycaemia, liver failure, cardiomyopathy, and sudden death. In young adults, CPT-II deficiency classically causes recurrent episodes of fasting or exercise induced muscle pain, rhabdomyolysis, and paroxysmal myoglobinuria, which in some cases may lead to renal failure. Although the enzyme defect is expressed in all tissues, the clinical manifestations are usually restricted to skeletal muscle. Since the first description in 1973 more than 50 similar cases have been reported and CPT deficiency is thought to be the most common cause of hereditary myoglobinuria.
The isolation and molecular characterisation of the human CPT-II gene has facilitated the identification of disease causing mutations in this gene. Although there is considerable genotypic heterogeneity with 12 different mutations identified so far, (and F Taroni, unpublished data) one mutant allele (Ser113Leu) accounts for almost 60% of all CPT-II alleles in patients with CPT-II deficiency.20

Despite the progress in this area, the diagnosis of CPT deficiency is still mainly based on direct enzyme measurement. Previously, such studies were hindered by the need to use either muscle homogenates or mitochondria enriched fractions of cultured skin fibroblasts, which involves the large scale culture of fibroblasts. In this paper we describe a simple cell permeabilisation technique which allows the measurement of CPT activity in a small amount of cultured skin fibroblasts or peripheral blood cells and which we have used to diagnose CPT deficiency in three patients with abnormal fatty acid oxidation. A diet low in fat but supplemented with medium chain triglycerides is increasingly suggested for the treatment of patients with mitochondrial long chain fatty acid β-oxidation disorders. However, because of problems in the isolation of the purified enzymes, little is known about the substrate specificity of either of the human CPTs. We therefore studied the substrate specificity of both CPT-I and CPT-II in permeabilised fibroblasts from two of the above patients, to assess the usefulness of medium chain triglyceride therapy. Using acyl-CoA esters of chain length C6 to C22, CPT-II was found to have considerable activity with medium chain acyl-CoA esters. In view of this finding, we assessed the metabolic response after a medium chain triglyceride load in a patient with CPT-II deficiency. The results have implications for both the diagnosis and management of patients with CPT deficiency.

Materials and methods

CASE REPORTS

Patient 1
A 35 year old man, the eldest of three siblings of unrelated parents with no previous family history of neuromuscular disease, presented with a 30 year history of episodes of myalgia, fatigue, weakness, and painful muscle stiffness followed by the passing of dark urine. In childhood these episodes were usually provoked by intercurrent viral infections. From the age of 15 years the episodes occurred more often, became more severe, and were induced by prolonged exertion. Short term exercise did not cause any symptoms and he had no second wind phenomena. Over the years the patient noticed a gradual deterioration of his exercise tolerance and eventually had to restrict his physical activities, although ingestion of carbohydrates or alcohol before starting the exercise had some protective effect.

General medical and neurological examinations were unremarkable, in particular, neither power nor muscle bulk were reduced. Serum alanine aminotransferase was raised at 70 U/l (normal < 43); bilirubin and all other routine haematological and biochemical variables including thyroid function tests were normal. Creatine kinase activity, plasma cholesterol, and triglycerides were within normal limits. During a 24 hour fast the patient remained normoglycaemic and plasma ketone body concentrations (patient 0.6 mmol/l; fasting reference range 0.02–0.49 mmol/l), and free fatty acids (patient 0.6 mmol/l; fasting reference range 0.06–0.70 mmol/l) at the end of the fast were normal. No organic aciduria or myoglobinuria were detected. A forearm ischaemic lactate test showed a normal increase of venous lactate and ammonia. Nerve conduction studies, ECG, and EMG disclosed no abnormalities.

Patient 2
A 55 year old man was admitted for investigation of episodic myalgia, muscle stiffness, and weakness followed by myoglobinuria, which first started at the age of 6. Similar to patient 1, the episodes were usually precipitated by prolonged strenuous exercise or viral infections; however, there was no relation to fasting. He performed well in short burst exercise and had no second wind phenomena. The patient was otherwise in good health and there was no family history of neuromuscular disorders. Physical examination disclosed an overweight man and on general medical and neurological examination there were no pathological findings. Routine biochemical and haematological investigations, liver function tests, creatine kinase activity, and urinalysis were normal. Total plasma ketone bodies and plasma glucose after a 24 hour fast were within the control range and urinary organic acid analysis was normal. A forearm ischaemic exercise test showed a normal rise in plasma lactate and ammonia concentration. Nerve conduction studies and EMG were unremarkable. A muscle biopsy showed discrete myopathic changes with mild lipid storage.

Patient 3
The patient, a boy, was the second child born to healthy non-consanguineous parents after a full term uneventful pregnancy. At three days of age the infant was found unresponsive and flacid, and had hepatomegaly. Blood glucose was 1.9 mmol/l and was associated with metabolic acidosis, severe hyperkalaemia, and raised urea and creatinine which was thought to be due to prerenal failure. After glucose and electrolyte infusions his condition improved rapidly and he was discharged home at 11 days of age. In the next few months he remained slightly hypotonic, but psychomotor development was otherwise unremarkable and all laboratory parameters including renal and liver function tests returned to normal. At the age of 7 months, after an upper respiratory tract infection, the patient again developed increasing lethargy, hypotonia, and drowsiness, and had several seizures. On physical examination pronounced hepatomegaly, tachycardia, peripheral oedema, and cardiomegaly were
Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy

noted. Laboratory results showed: hypoglycaemia (plasma glucose 0·2 mmol/l); absent or only mild ketonuria; arterial pH 7-32; hyper-ammonaemia (ammonia 240 μmol/l); plasma potassium 6·3 mmol/l; transiently increased alanine aminotransferase and aspartate aminotransferase; raised urea (16·6 mmol/l); plasma insulin < 2 μU/l (normal < 20 μU/l); plasma cortisol > 2000 nmol/l (normal 280–700 nmol/l). Plasma total carnitine was raised (85 μmol/l; normal 34–54 μmol/l), with only 8% in the acylated form (normal 13%–28%). Plasma creatine kinase concentrations and urinary organic acid analysis were within normal limits on the several occasions that they were measured. Other investigations included a liver biopsy, which showed widespread macrovesicular and microvesicular fatty changes and swollen mitochondria. A muscle biopsy, cranial CT and MRI, EEG, and renal ultrasound were all normal. Medium chain and very long chain acyl CoA dehydrogenase, assayed in fibroblasts with the electron transfer flavoprotein linked fluorimetric assay,\(^5\) were normal. The patient continued to have similar episodes of encephalopathy and hypotonia associated with hypoglycaemia, which were usually provoked by a mild viral illness, but he was well in between episodes with a normal biochemical profile in plasma and urine. During these episodes chest radiographs confirmed a persistent cardiomegaly and ECG showed a right bundle branch block. Because of poor cardiac contractility treatment with diuretics and digoxin was initiated. The cardiac function improved and treatment was discontinued 12 months later. From the age of 2·5 years episodes became less severe and since the age of 5 years he has not experienced hypoglycaemia. Growth has been maintained between the 3rd and 10th centile and at the age of 13 the patient has an IQ within the normal range.

CHEMICALS
Acyl-CoA esters of various chain lengths were synthesised from the corresponding free fatty acid using the mixed anhydride method\(^6\) and were purified on SepPak C18 bond elut cartridges. L-(methyl-H)Carnitine was obtained from Amersham International, Little Chalfont, Buckinghamshire, UK. All other chemicals were purchased from Sigma Chemical Co, Poole, Dorset and were of highest possible purity.

CULTURE OF FIBROBLASTS
 Fibroblasts were cultured in minimal essential medium supplemented with 10% foetal bovine serum, 1% minimum essential vitamins, 0·1 mg/ml streptomycin, and 100 U/ml benzylpenicillin. Monolayers were harvested by trypsinisation and the cell pellet was washed twice with Dulbecco’s phosphate buffered saline.\(^2\)

PERMEABILISATION OF FIBROBLASTS
 The fibroblast pellet was suspended in isolation buffer (20 mM HEPES, 250 mM sucrose, 3 mM EDTA, pH 7·5) and then incubated for one minute at room temperature with an aqueous solution of histone type II AS at a final concentration of 3 mg/ml. The cells were then sedimented by centrifugation at 11 000 g for one minute and washed with isolation buffer. After recentrifugation at 11 000 g for one minute, the final cell pellet was resuspended in 300 μl isolation buffer.

FATTY ACID OXIDATION STUDIES
Fatty acid oxidation flux and intermediates were determined as previously described.\(^9\)

CPT ASSAYS
CPT-I activity was measured as described by Demaure et al\(^1\) except that permeabilised fibroblasts, rather than cell homogenates, were used for the determination of activity. Activity of CPT-II was assayed after disruption of the mitochondrial membranes by sonication on ice (MSE Soniprep, 3 × 5 seconds at 8μ amplitude, with a two minute interval between bursts). Protein was determined by the method of Peterson.\(^3\)

METABOLIC STUDIES
Medium chain triglyceride loading was performed with medium chain triglyceride emulsion (Crodamol, 0·5 g/kg body weight), after a 14 hour overnight fast. Plasma ketone body, glucose, and free fatty acid concentrations were determined using a centrifugal analyser.\(^1\) Urine specimens, collected four hours and eight hours after the medium chain triglyceride load, were analysed for organic acids by gas chromatography-mass spectrometry.\(^7\)

MOLECULAR STUDIES
Genomic DNA was prepared from fibroblasts as described.\(^1\) The Arg63Cys, Pro50His, and Asp553Asn mutations were screened for by polymerase chain reaction (PCR) following the previously reported protocols.\(^1\)\(^9\) A PCR based method for the rapid detection of the common Ser113Leu mutation has been previously reported.\(^1\) In the present study, however, we have improved the efficiency of the reported method by using a different restriction enzyme along with a different primer set. A 150 bp region encompassing the C854T mutation was amplified and an artificial PhMI site was introduced into the mutant sequence by substituting T847 with C in the mutagenic sense primer LEUPMI (5′-ACAGAATAACATACAAAGCCACATT-3′; nucleotides 828–852, substitution is underlined). The antisense primer LEUVIS (5′-CGTTACTTCCATTTGCGTGTTCCACC- 3′) was complementary to nucleotides +97 to +121 of CPT-II intron 3. After an initial denaturation step at 97°C for four minutes, PCR was carried out for 30 cycles of one minute at 95°C, one minute at 60°C, and one minute at 72°C. In the presence of the C854T transition, PhMI digestion generated two fragments, of 125 bp and 25 bp, that could be easily differentiated from the uncut 150 bp wild type fragment by electrophoresis on an ethidium bromide stained 6% polyacrylamide gel. Screening for an additional eight recently identified CPT-II muta-
Table 1  Oxidation of [U-14C] hexadecanoate by fibroblasts and peripheral blood cells

<table>
<thead>
<tr>
<th></th>
<th>Fibroblasts</th>
<th>Blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (mean (SD))</td>
<td>48.2 (5.9) n = 11</td>
<td>7.1 (2.3) n = 14</td>
</tr>
<tr>
<td>Patient 1</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>Patient 2</td>
<td>9.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Patient 3</td>
<td>6.4</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Results are expressed as nmol C2 units formed/min/U citrate synthase activity.

Table 1  Oxidation of [U-14C] hexadecanoate by fibroblasts and peripheral blood cells

Results

FIBROBLAST AND LEUCOCYTE FATTY ACID OXIDATION

Oxidation of uniformly labelled 14C palmitate was reduced to less than 20% of control values in peripheral blood cells from patient 1 and in fibroblasts from patients 2 and 3 (table 1). Radio-high pressure liquid chromatography (radio-HPLC) analysis of the acyl CoA and acylcarnitine esters formed in fibroblasts from patient 2 and 3 (fig 1B, 1C) and in peripheral blood cells from patient 1 (not shown) showed a lack of significant chain shortening, with palmitoyl-CoA and palmitoylcarnitine as the only detectable intermediates. Patient 2 accumulated large amounts of palmitoylcarnitine and only relatively small quantities of palmitoyl-CoA (fig 1B), whereas the reverse was found with patient 3 (fig 1C).

CPT ACTIVITY

Activity of CPT-I, identified by its inhibition by malonyl-CoA, was measured in permeabilised fibroblasts in which mitochondrial integrity was maintained. After sonication, CPT activity was determined again in the presence and absence of malonyl-CoA (200 μM). The non-malonyl-CoA inhibitable activity represents the activity of CPT-II. The failure to release any further CPT activity after

![Figure 1](http://jnnp.bmj.com/)  

Radio-HPLC analysis of acyl-CoA esters and acylcarnitine esters in permeabilised fibroblasts of (A) a healthy control, (B) patient 2, and (C) patient 3. Incubations with U-14C-palmitate were performed as described previously.

![Figure 2](http://jnnp.bmj.com/)  

Malonyl-CoA inhibition of CPT-activities in (A) CPT-II deficient and (B) CPT-I deficient fibroblasts. Permeabilisation and measurement of CPT activities were performed as described in materials and methods. □ and △ indicate permeabilised cells before sonication, ■ and ▲ after sonication.
Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy

Table 2  Acyltransferase activities in cultured fibroblasts

<table>
<thead>
<tr>
<th>CPT-I (substrate)</th>
<th>CPT-III (substrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8-CoA</td>
<td>C12-CoA</td>
</tr>
<tr>
<td>Controls (mean (SD))</td>
<td>n = 6</td>
</tr>
<tr>
<td>Patient 1</td>
<td>372 (479)</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Not done</td>
</tr>
<tr>
<td>Patient 3</td>
<td>63</td>
</tr>
</tbody>
</table>

Acyltransferase activities were measured in duplicate and are expressed as pmol acylcarnitine formed/min/mg protein. CPT-I represents the malonyl-CoA sensitive and CPT-III the malonyl-CoA insensitive acyltransferase activity.

Table 3  CPT activities of peripheral blood cells

<table>
<thead>
<tr>
<th>CPT-1</th>
<th>CPT-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>163</td>
</tr>
<tr>
<td>Control 2</td>
<td>228</td>
</tr>
<tr>
<td>Patient 1</td>
<td>131</td>
</tr>
</tbody>
</table>

CPT activities are expressed as pmol palmitoylcarnitine formed/min/mg protein.

Sonication of CPT-II deficient cell lines confirmed that the cells were fully permeable to substrate after incubation with histone (fig 2A). In addition, the lack of measurable CPT-II activity in the CPT-I deficient patient before sonication proved that the mitochondria remained intact under our permeabilisation conditions (fig 2B).

Activity of CPT-I was normal with all substrates in fibroblasts from patients 1 and 2, whereas CPT-II activity was reduced to 40% for octanoyl-CoA (C8), to 5% for lauroyl-CoA (C12), and to 7% for palmitoyl-CoA (C16; table 2). Conversely, CPT-II activity was within the normal range in fibroblasts from patient 3, but CPT-I activity was clearly impaired for all three substrates, the deficiency again being more pronounced with long chain than with medium chain substrates (17% of controls for octanoyl CoA, 4% for lauroyl CoA, and 13% for palmitoyl-CoA) (table 2). CPT-II deficiency could also be demonstrated in permeabilised peripheral blood cells from patient 1 (table 3).

Molecular studies in the patients with CPT-II deficiency

Patient 2 was found to be heterozygous for the common Ser13Leu mutation,20 whereas patient 1 was heterozygous for the rarer Pro50His mutation.11 In both cases, the disease causing mutation on the other allele was not one of the known mutations and has not yet been identified. Both patients had the intragenic polymorphism Val368Ile and patient 2 was also positive for the Met647Val polymorphism.10

Substrate specificity profiles of CPT

We established a complete substrate specificity profile for all acyl-CoA substrates of chain length C6 to C22 in two controls and in patients with CPT-I and CPT-II deficiency. The profile for the malonyl-CoA sensitive CPT (fig 3A) shows an identical pattern for the controls and the CPT-II deficient patient, again confirming the validity of our permeabilisation procedure. CPT-I was most active with C12-CoA, the activity with C16-CoA being only about 40% of that with C12-CoA. There was no appreciable activity with acyl-CoA substrates of chain length C20 or longer. A second activity peak separate from the C12-CoA peak was seen with C8-CoA. The considerable loss of acyltransferase activity with C8-CoA in the CPT-I deficient patient compared with the controls indicates that most of the malonyl-CoA sensitive acyltransferase activity towards this substrate must be due to CPT-I. However, in the CPT-I deficient patient there is still some residual activity towards C8-CoA suggesting the existence of another malonyl-CoA sensitive enzyme.

![Figure 3](http://jnnp.bmj.com/)

**Figure 3** Specificity of cellular carnitine acyltransferase activities for acyl-CoA of various chain lengths in permeabilised fibroblasts (A) before sonication and (B) after sonication. Permeabilisation and enzyme assays were performed as described under materials and methods. CPT-I represents malonyl-CoA sensitive acyltransferase activity, CPT-II represents malonyl-CoA insensitive acyltransferase activity, and indicate controls, • indicates CPT-II deficient cell line; □ indicates CPT-I deficient cell line.
Figure 4  Time course of plasma ketone body concentrations after medium chain triglyceride loading. The loading was performed as described under materials and methods in a CPT-II deficient patient (■) and in controls (○; △). Ketone bodies are the sum of 3-hydroxybutyrate and acetoacetate.

The substrate specificities of the malonyl-CoA insensitive acyltransferases (fig 3B) were similar to those of the malonyl-CoA sensitive enzymes, with the highest activity being towards C12-CoA. A malonyl-CoA insensitive carnitine acyltransferase activity was found with C8-CoA in the CPT-II deficient patient. However, the large difference in residual medium chain activity between the CPT-II deficient fibroblasts and the controls suggests that a considerable proportion of the total transferase activity towards medium chain substrates is contributed by CPT-II.

METABOLIC STUDIES IN A PATIENT WITH CPT-II DEFICIENCY
In view of the substantial contribution of both mitochondrial CPTs towards medium chain acyltransferase activity, we decided to determine the efficacy of medium chain triglyceride supplements for patients with CPT deficiency. We therefore administered medium chain triglycerides to one of our patients with CPT-II deficiency (patient 1) and compared the results with those in controls. The time course of plasma ketone body concentrations (fig 4) was similar in the patient and controls after medium chain triglyceride loading, but by contrast with both controls, who only had a mild ketonuria, the patient developed a significant transient medium chain dicarboxylic aciduria.

Discussion
The typical clinical presentation of patients with CPT-II deficiency is of muscle pain and myoglobinuria after exhaustive exercise or fasting. Very occasionally, lipid accumulation can be shown in muscle biopsies, but by contrast with many other β-oxidation defects, serum carnitine concentrations are invariably normal and there is no characteristic organic aciduria. Although the enzyme is thought to be present in all tissues, a generalised metabolic disturbance is not found in adult type CPT-II deficiency, suggesting that hepatic fatty acid oxidation is relatively unaffected. This was true for patient 1 who remained normoglycaemic and had a normal ketotic response after a 24 hour fast. By contrast, the prominent clinical features in CPT-I deficiency are hepatic dysfunction, hypoketotic hypoglycaemia, and encephalopathy, but usually without myopathy or cardiomyopathy.10 11 This clinical pattern of selective tissue involvement is reflected biochemically by the presence of an enzyme defect only in liver and fibroblasts, but not in muscle.12 It was therefore concluded that liver and muscle specific isoforms of CPT-I exist. Recently Wei et al demonstrated two immunologically distinct isoforms of CPT-I in rat liver and muscle and found both isoforms expressed together in the heart. Assuming similar conditions in human tissues, all CPT-I deficient patients reported so far would have an abnormality of the liver isoform causing the predominant hepatic dysfunction. If, as in rats, “liver CPT-I” contributes less than 10% to the total cardiac CPT-I activity in humans,10 a cardiomyopathy would not be expected in these patients. However, patient 3 had clinical evidence of both liver and cardiac involvement. There are several possibilities to explain these findings: (1) the distribution of tissue specific isoforms of CPT-I is different in humans and rats; (2) the patient has a deficiency of both CPT-I isoforms; (3) the cardiac insufficiency was secondary to the severe metabolic disturbance. We think that the third possibility is the most likely as full cardiac recovery has been seen.

In all three patients measurement of β-oxidation flux and acylcarnitine intermediates strongly suggested CPT deficiency. The much reduced β-oxidation flux indicated a defect of long chain fatty acid oxidation and the complete absence of chain shortened intermediates allows differentiation between a CPT and a very long chain acyl-CoA dehydrogenase deficieny or electron transfer flavoprotein deficiency.9 High concentrations of palmitoylcarnitine compared with palmitoyl-CoA (fig 1B) indicate a lack of CPT-II activity, whereas large amounts of palmitoyl-CoA relative to palmitoylcarnitine are seen in CPT-I deficiency (fig 1C).

Selective permeabilisation with histone II-AS preserves mitochondrial integrity and thus allows clear distinction of mitochondrial CPT-I and CPT-II activities in fibroblasts and peripheral blood cells from controls and patients (tables 2 and 3). Both patient 1 and patient 2 were diagnosed by this new method. Using histone permeabilised peripheral blood cells, a definitive enzymatic diagnosis of CPT deficiency can therefore be made from 10 ml blood within a few hours and without subjecting the patient to a muscle biopsy. Indeed, because of the greater proportion of CPT-II activity (66% of total CPT activity is due to CPT-II) peripheral blood cells represent a better tissue for the diagnosis of CPT-II deficiency than fibroblasts (43% of total CPT activity is due to CPT-II; tables 2 and 3).

The relative simplicity of our modified CPT assay enabled us to determine a complete substrate specificity profile for cellular CPT activities in human fibroblast cell lines. Surprisingly, the highest CPT activity is seen
Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy

not with long chain substrates, but with C12-CoA, and there is virtually no activity towards acyl-CoA esters of chain length C20 or more. C12-CoA is therefore a more appropriate substrate for the diagnosis of CPT deficiency than the commonly used C16-CoA.

Whereas the genetic basis of CPT I deficiency remains to be elucidated, some disease causing mutations have been characterised in patients with CPT-II deficiency. Although a common Ser113Leu transition is found in 60% of patients with CPT-II deficiency, a considerable proportion of patients have other mutations. Both CPT-II deficient patients described in this report had different disease causing mutations, thus emphasising the molecular heterogeneity of this disorder. It is interesting to note that in both cases, the identified mutations were associated with the same haplotype (the Ile368-Val647 for the Ser113Leu mutations and the Ile368-Met647 for the Pro50HiS mutation) as that found in all other patients carrying either of the two mutations. This suggests that despite different ethnic origins (southern European ancestry and English ancestry in our patients) both mutations seem to derive from the same ancestral mutated allele.

Medium chain triglycerides have been used successfully in the treatment of mitochondrial long chain fatty acid oxidation disorders and are sometimes recommended for CPT deficiency.23 Despite the major contribution of CPT-II towards total medium chain acyltransferase activity in vitro (fig 3) the ketotic response of a CPT-II deficient patient in vivo to a medium chain triglyceride load was normal (fig 4). This confirms the existence of a carnitine independent mitochondrial uptake mechanism for medium chain fatty acids which must have sufficient capacity to allow for adequate ketogenesis. However, the appearance of a medium chain dicarboxylic aciduria in the patient, but not in the controls, after medium chain triglyceride loading suggests that CPT also plays a part in transferring medium chain fatty acids into the mitochondrial compartment. If the capacity of the direct (carnitine independent) uptake mechanism is exceeded in the CPT deficient patient, then the extramitochondrial pathways (peroxisomal and \( \omega \)-oxidation) will be utilised to clear the excess medium chain fatty acids leading to the production of diacylglycerol fatty acids.

Medium chain triglycerides in a dose of 0.5 g/kg body weight, which is less than that used by other investigators, probably overload the direct uptake mechanism. Although medium chain supplements to a low fat diet can certainly improve ketone body production, we recommend a careful adjustment of the dose in order not to exceed the limited CPT independent transport capacity for medium chain fatty acids. Also, as C12-CoA is the optimum substrate for CPT, medium chain triglyceride preparations with a high proportion of C12-fatty acids should be avoided in the treatment of these patients.

This study was supported by grants from the Wellcome Trust and the Northern Regional Health Authority, UK and from Telethon-Italia (No 553 to FT). J S is a recipient of a research fellowship from the Deutsche Forschungsgemeinschaft.

We acknowledge the expert technical assistance of Mr Alasir Sinn in measuring plasma ketone bodies and free fatty acids.

We are grateful to Dr Peter Hodgson and Dr David Stevens for referring patients 1 and 2 respectively. We are also grateful to Dr Rodney Pollett for allowing us to study the cell line from patient 3.

26. Glasgow AM, Engel AG, Bier DM, et al. Hypoglycaemia,


Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy.

J Schaefer, S Jackson, F Taroni, P Swift and D M Turnbull

J Neurol Neurosurg Psychiatry 1997 62: 169-176
doi: 10.1136/jnnp.62.2.169

Updated information and services can be found at:
http://jnnp.bmj.com/content/62/2/169

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/