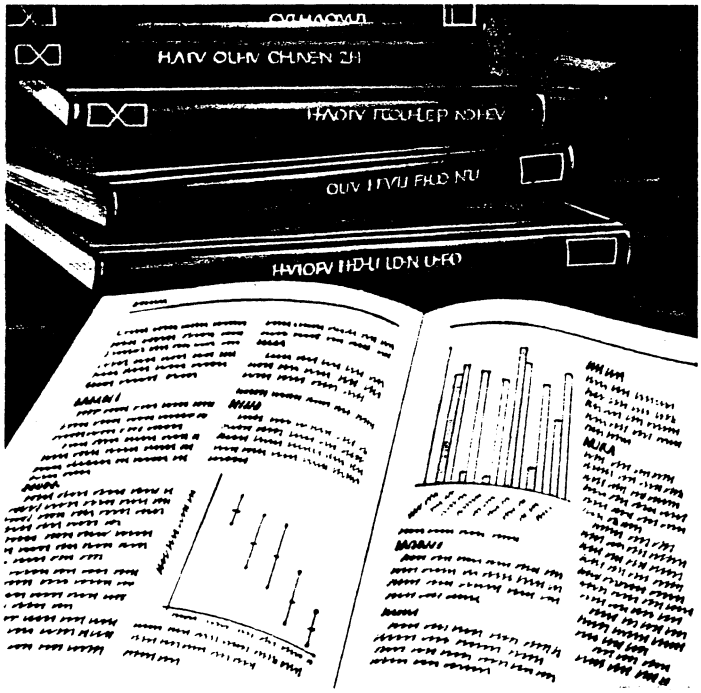

L-CARNITINE IN THE MANAGEMENT OF METABOLIC DISORDERS IN CHILDREN

A review of key literature

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Ronald Chalmers is Senior Lecturer in paediatric Metabolism at St George's Hospital Medical School, University of London and Honorary Consultant Biochemist to St George's Healthcare. Following initial research at St Bartholomew's Hospital Medical School on disorders of purine metabolism he moved in 1970 to the Medical Research Council's new Clinical Research Centre at Northwick Park Hospital. There he obtained his PhD in 1974 for research on the emerging organic acidurias and remained on the Scientific Staff of the Medical Research Council at the CRC to continue his research on metabolic diseases and on the use of erythrocytes as drug and enzyme carriers. He established an international reputation in these areas and was awarded a DSc in recognition of his work in 1990. He joined the staff of St. George's Hospital Medical School in 1992 where, with Dr Bain, he has established the Paediatric Metabolism Unit in the Department of Child Health. He has now been involved in research into metabolic diseases and intermediary metabolism for some 30 years, maintaining a particular interest in disorders of organic acid and acyl group metabolism. As part of this work he has been at the forefront of research into the metabolism and functions of L-carnitine and, in a collaborative group at the CRC, the first in the U.K to use L-carnitine in the therapy of patients with these metabolic diseases. Dr. Chalmers is author/co-author of more than 150 research papers in the fields of analytical chemistry, biochemistry and metabolic disorders and of a major text ("Organic Acids in Man") and book chapters on disorders of organic acid metabolism.

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Murray Bain is Senior Lecturer and Consultant Paediatrician in Child Health (with a special interest in metabolic disease) at St George's Hospital Medical School, University of London and St George's Healthcare. He is a graduate of Edinburgh University, with an honours degree in biochemistry. He obtained his MD by research into *in vivo* placental transport mechanisms while with the Medical Research Council at the Clinical Research Centre and Northwick Park Hospital where he was also involved in the care of children with metabolic disorders. He extended his placental research on appointment to St George's and developed his major interest in paediatric metabolic disease. Together with Dr. Charmers, he has established the Paediatric Metabolism Unit in the Department of Child Health. He has now been involved in both the clinical care and research on patients with a wide variety of disorders of intermediary metabolism for more than eight years, with a particular interest in developing innovative approaches to treatment including the use of antibiotics in disorders of propionate metabolism, the clinical metabolic use of L-carnitine and the use of growth hormone to promote anabolism in metabolic disorders. Dr. Bain is author/co-author of some 20 clinical and research papers in these areas.

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THE MAJOR ORGANIC ACIDURIAS

Introduction

Disorders of organic acid metabolism, more commonly referred to as organic acidurias or organic acidaemias, comprise a diverse group of diseases whose biochemistry encompasses several areas of intermediary metabolism. These include metabolic pathways associated with amino acid metabolism, fatty acid metabolism and ketogenesis and also of pyruvate and carbohydrate metabolism including the tricarboxylic acid (TCA or Krebs) cycle. Such disorders may occur in or involve many different organelles including the mitochondria, the cytosol, peroxisomes and microsomes. These disorders share the common features that they are characterised by the accumulation in body tissues and fluids, particularly urine, of organic acids and their acyl esters and conjugates. The latter include conjugates with L-carnitine to form basic acylcarnitine esters.

In addition to the common features of chemistry and biochemistry these disorders of intermediary metabolism also share some common clinical characteristics. The patients frequently present with acute symptoms in early life with acidosis, ketosis, hypoglycaemia and/or hyperammonaemia associated with vomiting, convulsions and coma and Reye's syndrome-like attacks after a previous uneventful history. The disorders are often lethal in the newborn and young child and survivors may be physically or mentally handicapped. Other patients may present later in childhood with established failure to thrive, neurological deterioration or with sudden acute and profound attacks associated with infections and trauma. Although the incidence of individual diseases may be low, collectively their overall incidence may be as high as 1:3000 liveborn infants. Early and precise diagnosis is essential since, when coupled with adequate early therapy, greatly improved prognosis with normal subsequent physical and mental development may result.

The immediate therapeutic measures undertaken, often before a precise diagnosis has been made, are generally common to all or most organic acidurias, irrespective of the primary defect: they involve management of the acute episodes by supportive therapy [e.g. assisted ventilation, control of acidosis, restriction or cessation of protein intake accompanied by oral or i.v. glucose to maintain calories and counter hypoglycaemia, and increased fluid (saline) intake to counter dehydration and electrolyte loss], accompanied by detoxification measures to remove any accumulating metabolites and potentially toxic intermediates (e.g. exchange

transfusions, peritoneal dialysis or forced diuresis). Once a more specific diagnosis has been established, more specific and individual therapeutic measures suited to particular disorders may be instituted. These may involve environmental manipulation by reducing the load of substrate presented to the mutant or absent enzyme, including the use of dietary restriction, inhibition of substrate synthesis, substrate removal and coenzyme (vitamin) supplementation at pharmacological levels. Substrate removal is also an effective means of therapy in some organic acidurias, for example in isovaleric acidaemia isovaleryl groups are effectively conjugated with glycine or L-carnitine.

Many organic acidurias are associated with the accumulation in tissues of acyl CoA esters with consequent toxic effects due directly to the acyl CoA esters and to metabolites produced by alternative pathways. Among the secondary effects of acyl CoA accumulation is the sequestration of available free CoA, resulting in severe inhibition of CoA-dependent metabolic processes including pyruvate oxidation, the tricarboxylic acid cycle and β-oxidation, resulting in secondary lactic acidosis and dicarboxylic aciduria. L-carnitine (4-trimethylamino-3-hydroxybutyrate) is important in the modulation of the availability of free CoA and may exchange readily with acyl CoA esters to form acylcarnitine esters via the action of carnitine acyltransferases. This occurs in many of the organic acidurias, presumably as an attempt to detoxify acyl CoA esters accumulating in the mitochondria and increasing the removal of acyl moieties into the urine. As a consequence, patients with many organic acidurias exhibit increased acylcarnitine excretion with consequent L-carnitine insufficiency or deficiency and L-carnitine has been advocated and used in both the acute and long-term treatment of patients with these diseases. L-carnitine has been of particular value in the treatment of patients with propionic acidaemia, methylmalonic aciduria and isovaleric acidaemia and in the acute treatment of patients with medium-chain acyl CoA dehydrogenase deficiency and with 3-hydroxy-3-methylglutaric aciduria. The efficacy of L-carnitine as a therapeutic measure in the organic acidurias is gaining acceptance because of proven clinical and biochemical effects but its use requires careful assessment in each disorder concerned and the individual patient's response to therapy also requires careful monitoring.

1. Methylmalonic Aciduria (MMA)

The branched-chain amino acids L-isoleucine and L-valine are metabolised via a sequence of metabolic pathways to propionyl CoA. Other precursors of propionyl CoA include methionine, threonine, cholesterol and odd-carbon number fatty acids. Propionate is also produced normally by gut bacteria in substantial quantities, which is then absorbed from the colon and utilised via conversion into propionyl CoA. Propionyl CoA is subsequently metabolised via the action of propionyl CoA carboxylase to methylmalonyl CoA, and thence to succinyl CoA and into the TCA cycle. When the further metabolism of methylmalonyl CoA is blocked (by deficient activity of B_{12}-dependent methylmalonyl CoA mutase), methylmalonic aciduria (MMA) results. Many of the clinical and biochemical symptoms of MMA are secondary to accumulation of propionyl CoA. Patients show a greatly increased urinary excretion of methylmalonic acid accompanied by increased excretion of methylcitric acids, 3-hydroxypropionic acid and other metabolites of propionyl CoA. Methylmalonate concentrations in urine may be very great and increased concentrations are also observed in blood plasma and in cerebrospinal fluid. Some patients respond to pharmacological doses of vitamin B_{12} with concomitant major reduction of methylmalonate concentrations and of propionyl metabolites.

Patients with methylmalonic aciduria show greatly increased ratios of acylcarnitine concentrations to free carnitine concentrations in urine and blood in which the predominant acylcarnitine is propionylcarnitine, providing direct evidence for the accumulation of propionyl CoA and for secondary carnitine insufficiency in this disorder. Patients show favourable biochemical and clinical responses to administration of L-carnitine, both under basal metabolic conditions and during severe acute metabolic decompensation, with increased propionylcarnitine excretion and L-carnitine has a proven place in the treatment of these patients.

1.1 Roe CR. Hoppel CL. Stacey TE. Chalmers RA. Tracey BM. Millington DS. Metabolic response to carnitine in methylmalonic aciduria. Arch. Dis. Childh. 1983; 58: 916–920.

Authors' abstract: Patients with methylmalonic aciduria have an excessive intramitochondrial accumulation of acylcoenzyme A compounds that may reduce the availability of free coenzyme A (CoA) for normal metabolic requirements, producing profound metabolic disturbances. Giving carnitine to a patient with methylmalonic aciduria produced an increase in hippurate excretion [an index of intramitochondrial adenosine triphosphate (ATP) and CoA availability], a large increase in short chain urinary acylcarnitines, and a reduction in excretion of methylmalonate and methylcitrate. These acylcarnitines were shown by fast atom bombardment and B/E linked scan mass spectrometry to be propionylcarnitine and acetylcarnitine. L-carnitine acts by removing (detoxifying) propionyl groups, thereby releasing CoA and restoring ATP biosynthesis and concentrations towards normal. L-carnitine may play a central role in maintenance of mitochondrial and cellular homoeostasis in methylmalonic aciduria and propionic acidaemia. These principles may provide an approach to the treatment of this and other disorders, inherited and acquired, in which accumulation of acyl CoA metabolites results in sequestration of free CoA, thereby perturbing metabolic homoeostasis.

COMMENT:

This paper was one of the earliest to provide a scientific basis for the biochemical and clinical benefits of L-carnitine therapy in an organic aciduria (methylmalonic aciduria). It introduced the concept of mitochondrial 'toxicity' (on energy metabolism) that can result from inherited metabolic disease and that can be alleviated by supplementary L-carnitine. A corollary is that plasma carnitine levels may not always be a direct reflection of either intra-mitochondrial levels or metabolic requirements.

1.2 DiDonato S, Rimoldi M, Garavaglia B, Uziel G. Propionylcarnitine excretion in propionic and methylmalonic acidurias: a cause of carnitine deficiency. Clin. Chim. Acta 1984; 139: 13–21.

Authors' abstract: Two patients with propionic acidemia (PA) and two patients with methylmalonic aciduria (MMA) had low plasma free carnitine and increased short-chain acylcarnitines. Urinary excretion of free carnitine was decreased, while the excretion of short-chain acylcarnitines, mostly propionylcarnitine, was increased. Carnitine supplementation markedly increased the short-chain acylcarnitine fractions of both plasma and urine. Total carnitine content was decreased in skeletal muscle biopsies obtained from two of the patients. It is suggested that in these organic acidurias mitochondrial propionylcarnitine, formed from free carnitine and excess propionylCoA, exchanges with free cytosolic carnitine: propionylcarnitine is then lost in the urine, causing secondary carnitine deficiency in the tissues.

COMMENT:

An early paper supporting the aetiology of the carnitine deficiency found in MMA and PA as due to loss of free carnitine as the conjugate propionylcarnitine in urine. Biochemical and clinical improvements were seen with L-carnitine therapy, but episodes of metabolic acidosis continued (no mention of whether more or less often) and 1 child died despite (maintenance) L-carnitine therapy. L-carnitine therapy can make significant contributions to the maintenance of metabolic stability in these conditions, but it should not be regarded as a panacea.

1.3 Penn D, Schmidt H, Otten A, Schmidt-Sommerfeld E. Carnitine in the treatment of methylmalonic aciduria. Monats. Kinderh. 1986; 134: 758–761.

Authors' abstract: Carnitine metabolism was studied and a therapeutic trial with L-carnitine was undertaken in 3 patients with methylmalonic aciduria. Prior to carnitine therapy, the concentration of free carnitine was diminished and the contribution of acylated carnitine to total carnitine was increased in both plasma and urine. During a metabolic crisis in a patient the intravenous administration of L-carnitine greatly increased, the urinary excretion of acylcarnitine and the plasma concentration of methylmalonic acid fell. In all 3 patients, the chronic oral administration of L-carnitine resulted in the normalisation of the plasma free carnitine concentrations and an increased urinary excretion of carnitine esters. One patient clearly showed clinical improvement under carnitine therapy. The administration of L-carnitine to patients with methylmalonic aciduria results in an increased elimination of toxic propionyl groups and thus to a regeneration of intramitochondrial CoA. In conjunction with appropriate dietary measures, this may improve the metabolic situation of these patients.

COMMENT:

This paper provides evidence of biochemical benefits from L-carnitine therapy, but evidence of clinical benefit in 1 patient only.

1.4 Chalmers RA. Current research in the organic acidurias. J. Inher. Metab. Dis. 1989; 12: 225–239.

No Author's abstract [Special edition].

COMMENT:

A useful review of one centre's experience studying L-carnitine metabolism and therapy in inherited metabolic disease. It follows on from 1.1 above, and provides more data, but concentrates on organic acidurias and basic biochemical studies and techniques. There is also only a brief reference to the wide range of metabolic conditions for which L-carnitine therapy is worthy of exploration.

2. Propionic Acidaemia (PA)

The branched-chain amino acids L-isoleucine and L-valine are metabolised via a sequence of metabolic pathways to propionyl CoA. Other precursors of propionyl CoA include methionine, threonine, cholesterol and odd-carbon number fatty acids. Propionate is also produced normally by gut bacteria in substantial quantities, which is then absorbed from the colon and utilised via conversion into propionyl CoA. Propionyl CoA is subsequently metabolised via the action of propionyl CoA carboxylase to methylmalonyl CoA, and thence to succinyl CoA and into the TCA cycle. Propionic acidaemia is caused by deficient activity of propionyl CoA carboxylase, propionyl CoA accumulates and is metabolised alternatively, causing most of the clinical and biochemical features of the disease.

Patients with propionic acidaemia present with severe ketoacidosis, marked hypotonia, areflexia, vomiting, hyperventilation, apnoea, lethargy and coma, usually in early infancy. Hypoglycaemia and hyperammonaemia with neutropenia, thrombocytopenia and pancytopenia may occur and classically the disorder is associated with hyperglycinaemia (ketotic hyperglycinaemia), although this is not observed in all cases. The disorder is difficult to control and most patients die during acute episodes with survivors frequently showing permanent neurological damage. The clinical spectrum observed is illustrative of the heterogeneity of the disease.

Patients with propionic acidaemia consistently show increased excretion of methylcitric acids, generally associated with 3-hydroxypropionate and other metabolites of propionyl CoA including, particularly, propionylglycine and 3-hydroxy-n-valerate. Odd-carbon number fatty acids accumulate and form abnormal triglycerides and lipids in brain and other tissues. Secondary inhibition of other mitochondrial enzyme systems by propionyl CoA also occurs, for example inhibition of N-acetylglutamate synthesis probably underlying the hyperammonaemia observed, and is also the cause of the secondary hypoglycaemia and hyperglycinaemia in patients with the disorder. More direct evidence of the accumulation of propionyl CoA in propionic acidaemia has been presented by the observation of propionylcarnitine as the major acylcarnitine present in increased concentrations in urine, since propionylcarnitine can only be formed by transfer of the acyl moiety from propionyl CoA. Administration of exogenous L-carnitine to patients with the disorder results in an increase in the excreted amounts of propionylcarnitine and thus L-carnitine has value in the treatment of propionic acidaemia.

2.1 Kurczynski TW, Hoppel CL, Goldblatt PJ, Gunning WT. Metabolic studies of carnitine in a child with propionic acidemia. Pediatr. Res. 1989; 26: 63–66.

Authors' abstract: Carnitine metabolism was studied in a 7-yr-old boy with propionic acidemia due to an almost total deficiency of propionyl-CoA carboxylase. The initial diagnosis was made at 3 wks of age followed by numerous episodes of metabolic acidosis despite a low-content branch-chain amino acid diet containing supplemental biotin. Although clinically stable and in a nonacidotic state, the plasma concentration of total carnitine was normal (38.9 microM; normal = 46 +/- 10, mean +/- SD, n = 30) whereas free carnitine was decreased (5.7 microM; normal = 37 +/- 8) and short-chain acylcarnitines were increased (28.6 microM; normal = 5.7 +/- 3.5). Skeletal muscle and liver specimens obtained at open biopsy had low total and free carnitine contents and increased ratio of short-chain acylcarnitines to free carnitine. Short-chain acylcarnitine content was low in liver but increased in skeletal muscle. The liver contained fatty vacuoles, enlarged mitochondria with paracrystalline inclusions, and numerous peroxisomes whereas the skeletal muscle also had lipid vacuoles and an increase in number and size of mitochondria. A carnitine challenge test (100 mg L-carnitine/kg body wt via a gastrostomy tube) resulted in a peak plasma carnitine concentration at 120 min. With maintenance therapy of 100 mg L-carnitine/kg/day the plasma free carnitine remained relatively low, the plasma glycine concentration decreased, and urinary acylcarnitine excretion increased. This study demonstrates that the alterations in carnitine and its derivatives observed in plasma and urine reflect the same type of altered distribution in tissue and provides further data on the effects of L-carnitine therapy.

COMMENT:

This paper presents a single case study of the effects of L-carnitine in propionic acidaemia that includes pre-treatment liver and muscle biopsy data. It demonstrates structurally abnormal mitochondria to parallel the disordered biochemical function demonstrated in 1.1 above, and also the importance of measuring both free and acyl carnitine (short chain acylcarnitines referred to in the paper will be propionylcarnitine – see 2.3). Biochemical improvement was demonstrated with L-carnitine administration but there is no mention of any clinical benefit. Control ranges for biopsy data used patients with cardiomyopathy, but we now know that non-metabolic cardiomyopathy can increase free and total plasma carnitine by about 17%.

2.2 Shigamatsu Y, Mori I, Nakai A, Kikawa Y, Kuriyama M, Konishi Y, Fujii T, Sudo M. Acute infantile hemiplegia in a patient with propionic acidaemia. Eur. J. Paediatr. 1990; 149: 659–660.

Authors' abstract: A 10-month-old girl with mild developmental delay became hemiplegic after seizures. Cranial CT scan and magnetic resonance imaging (MRI) revealed no lesions related to vascular diseases, but brain atrophy on the right side was remarkable. Digital subtraction angiography showed slightly decreased visualization of peripheral branches of the right medial cerebral artery. Propionic acidaemia was diagnosed on the basis of high plasma levels of propionic acid and its metabolites and the elevated urinary excretion of these acids. With therapy, the levels of these acids fell, and her left hemiplegia disappeared 3 months later.

COMMENT:

A case report emphasising the variety of clinical presentations, in this case acute infantile hemiplegia, that may be seen with organic acidurias, and describing resolution of the hemiplegia with low protein/high calorie diet and L-carnitine supplements. The difference in metabolite profiles between CSF and plasma, presumably due to the non-homogeneous permeability profile of the blood brain barrier, (CSF:plasma ratio for methylcitrate 10–35, 0.25 for free carnitine) may partly explain the predilection for neurotoxicity in both propionic acidaemia and methylmalonic aciduria.

2.3 Davies SEC, Iles RA, Stacey TE, de Sousa C, Chalmers RA. Carnitine therapy and metabolism in the disorders of propionyl CoA metabolism studied using 1H-NMR spectroscopy. Clin. Chim. Acta 1991; 204: 263–278.

Authors' abstract: 1H-NMR spectroscopy has been used to study metabolic perturbations in patients with disorders of propionyl-CoA metabolism during the administration of oral and intravenous L-carnitine. The administration of L-carnitine either in the form of a challenge or as a therapeutic measure resulted in an increased excretion of propionylcarnitine, consistent with the removal of accumulated intramitochondrial propionyl-CoA esters. Additionally, during the therapeutic administration of L-carnitine excretion of acetylcarnitine occurred, coincident with an improvement in clinical condition and confirming the intracellular propionyl-CoA depletion. An additional benefit from the formation of acylcarnitines may be an accompanying intracellular alkalinisation.

COMMENT:

This study uses magnetic resonance spectroscopy (MRS) to replicate and further develop the data presented in 1.1 above. MRS though somewhat insensitive at present, allows measurement of organic acids, amino acids, and acylcarnitine conjugates in a single analysis. The 'mitochondrial toxicity' occurring in both MMA and PA is again demonstrated with its amelioration by L-carnitine therapy. Data on 3 patients are presented. In healthy control subjects, the predominant short-chain acylcarnitine is acetylcarnitine. Of an administered dose of L-carnitine only approximately 5% is absorbed, with 70% being excreted unchanged in the urine, and the remaining 30% appearing as acetylcarnitine. The authors of this paper demonstrate that in MMA and PA, propionylcarnitine predominates and acetylcarnitine only appears in the urine after carnitine administration, indicating more normal mitochondrial function.

2.4 DiDonato S, Rimoldi M, Garavaglia B, Uziel G. Propionylcarnitine excretion in propionic and methylmalonic acidurias: a cause of carnitine deficiency. Clin. Chim. Acta 1984; 139: 13–21.

Authors' abstract: Two patients with propionic acidemia (PA) and two patients with methylmalonic aciduria (MMA) had low plasma free carnitine and increased short-chain acylcarnitines. Urinary excretion of free carnitine was decreased, while the excretion of short-chain acylcarnitines, mostly propionylcarnitine, was increased. Carnitine supplementation markedly increased the short-chain acylcarnitine fractions of both plasma and urine. Total carnitine content was decreased in skeletal muscle biopsies obtained from two of the patients. It is suggested that in these organic acidurias mitochondrial propionylcarnitine, formed from free carnitine and excess propionyl CoA, exchanges with free cytosolic carnitine: propionylcarnitine is then lost in the urine, causing secondary carnitine deficiency in the tissues.

COMMENT:

An early paper supporting the aetiology of the carnitine deficiency found in MMA and PA as due to loss of free carnitine as the conjugate propionylcarnitine in urine. Biochemical and clinical improvements were seen with L-carnitine therapy, but episodes of metabolic acidosis continued (no mention of whether more or less often) and 1 child died despite (maintenance) L-carnitine therapy. L-carnitine therapy can make significant contributions to the maintenance of metabolic stability in these conditions, but it should not be regarded as a panacea.

2.5 Massoud AF, Leonard JV. Cardiomyopathy in propionic acidaemia. Eur. J. Paediatr. 1993; 152: 441–445.

Authors' abstract: Following the death of a patient with propionic acidaemia with a cardiomyopathy, we reviewed 19 patients with the same disorder for evidence of cardiomyopathy. Six patients were found to meet the diagnostic criteria. Three patients died and in the other three the cardiac disease resolved completely. All patients were treated with standard therapy and some received L-carnitine but this did not seem to influence the eventual outcome. Cardiomyopathy is an important complication of propionic acidaemia and may be rapidly fatal.

COMMENT:

This paper documents cardiomyopathy as a now recognisable association with propionic acidaemia. It is a retrospective review of 6 cases seen over about 27 years at one institution, but clinically managed at some 4 different hospitals. As such the quality of the clinical data is quite variable, and attributing a precise and uniform causality for the cardiomyopathy given the different clinical and pathological presentations is fraught with uncertainty. 4 patients received L-carnitine therapy: one recovered completely; one died from progressive cardiac failure; one collapsed and died in association with intubation for 'severe bronchospasm and cyanosis;' and the fourth patient collapsed some 20 hours after resuscitation for a dislodged central venous catheter with only mild ventricular dysfunction on echocardiography. Both patients not given L-carnitine recovered, though in one there was still echocardiographically detectable cardiomyopathy 2 months after it was first detected. Cardiomyopathy may be a new complication of propionic acidaemia but there are inadequate data in this paper on which to justify a definitive assessment of the efficacy of L-carnitine on cardiomyopathy.

3. Isovaleric Acidaemia (IVA)

Isovaleric acidaemia is caused by deficient activity of the specific FAD and ETF-dependent isovaleryl CoA dehydrogenase in the metabolism of L-leucine. This leads to intracellular accumulation of isovaleryl CoA with the appearance of characteristic metabolites in body fluids and of a characteristic odour of sweaty feet or cheese during acute episodes. The disorder is characterised clinically by episodes of acidosis, vomiting, ataxia and tremors that may progress to lethargy and coma. Many patients present acutely in the newborn period with severe metabolic acidosis and ketosis, lethargy and severe neurological symptoms including convulsions and coma. Death has occurred within a few weeks in about half of such cases with overwhelming sepsis, leucopenia and pancytopenia contributing to the high mortality. Patients who survive this initial acute period show a subsequent course analogous to those presenting later in life and the differences in presentation are a reflection of the spectrum of disease associated with residual enzyme activity, environmental factors and the modifying effects of different enzymatic phenotypes in different individuals. Prognosis with effective treatment, particularly during the first 1–2 years of life, is good.

The accumulating isovaleryl CoA is metabolised to 3-hydroxy-isovaleric acid and, via conjugation through the action of glycine-N-acylase, to isovalerylglycine, the latter being the most characteristic metabolite and always present in urine from affected patients. The characteristic odour is due to isovaleric acid itself which only accumulates during acute episodes. The free isovaleric acid that accumulates in body fluids, including CSF, during acute ketoacidotic episodes shows an encephalopathic action in experimental animals and is probably the cause of the coma, in particular, and may be responsible for the mild neurological damage observed in some patients. Treatment of acute episodes in isovaleric acidaemia may require detoxification by peritoneal dialysis or exchange transfusions and dietary management with a moderately reduced protein intake is also effective for longer term care. The observation that glycine-N-acylase has a relatively high affinity for isovaleryl groups, leading to the increased excretion of isovalerylglycine in isovaleric acidaemia, prompted the use of supplemental glycine therapy in the disease. This may be of particular benefit during acute ketoacidotic episodes but may be of less benefit for long-term management in preventing further attacks.

Patients with isovaleric acidaemia consistently show reduced free carnitine concentrations in plasma with greatly increased isovalerylcarnitine excretion into urine. Supplemental L-carnitine has been used in the therapy of isovaleric acidaemia both to augment detoxification of isovaleryl groups

by formation of isovalerylcarnitine, and thereby to restore mitochondrial homeostasis by release of free CoA and increased ATP synthesis, and through the latter processes also to increase further the formation and excretion of isovalerylglycine.

3.1 Roe CR, Millington DS, Maltby DA, Kahler SG, Bohan TP. L-carnitine therapy in isovaleric acidemia. J. Clin. Invest. 1984; 74: 2290–2295.

Authors' abstract: Isovaleric acidemia, resulting from isovaleryl-coenzyme A dehydrogenase deficiency, is associated with marked reduction of free carnitine in both plasma and urine. Fast atom bombardment-mass spectrometry, hydrolysis, and gas chromatography/mass spectrometry have unequivocally identified the existence of isovalerylcarnitine, a new metabolite specific for this disorder. Administration of equimolar amounts of glycine or L-carnitine separately with leucine demonstrated that isovaleryl-coenzyme A is removed by supplemental L-carnitine in the form of isovalerylcarnitine as effectively as it is by glycine, in the form of isovalerylglycine. When L-carnitine is given alone, excretion of isovalerylglycine decreases in preference to enhanced excretion of isovalerylcarnitine and hippurate. Treatment with L-carnitine alone has proven effective in preventing further hospitalizations in a patient with this genetic disorder.

COMMENT:

This is a single case study demonstrating oral glycine and L-carnitine to be equally effective at eliminating the accumulated isovaleryl CoA through conjugation and subsequent renal excretion. L-carnitine was more effective at lowering plasma levels of isovaleric acid than therapy with glycine, and increased the efficiency of glycine conjugation through improved mitochondrial homeostasis. The value of combination therapy with both carnitine and glycine was not explored.

3.2 De Sousa C, Chalmers RA, Stacey TE, Tracey BM, Weaver CM, Bradley D. The response to L-carnitine and glycine therapy in isovaleric acidaemia. Eur. J. Paediatr. 1986; 144: 451–456.

Authors' abstract: The profound metabolic disturbances which occur in isovaleric acidaemia are due to the intramitochondrial accumulation of isovaleryl coenzyme A (CoA) with a consequent reduction in the availability of free CoA. Secondary carnitine insufficiency is also a feature of this and other disorders of organic acid metabolism. A patient who presented at 2.5 years of age was diagnosed using GC-MS as having isovaleric acidaemia. She showed the full spectrum of abnormal organic acids previously associated with the 'neonatal' form of the disease despite her late presentation, indicating that it is inappropriate to refer to acute early and late onset forms of isovaleric acidaemia. Instead, a spectrum of disease exists, determined by environmental factors, residual enzyme activities and modifying effects of different phenotypes in different individuals. She also showed evidence of carnitine insufficiency. An oral challenge with L-carnitine resulted in the excretion of large amounts of urinary acylcarnitines which were shown by use of fast atom bombardment mass spectrometry to be primarily isovalerylcarnitine. Regular glycine supplementation caused no significant increase in urinary isovalerylglycine and had to be stopped because of side-effects after 5 days. An oral L-carnitine challenge during glycine supplementation resulted in a marked increase in isovalerylglycine excretion, again associated with the excretion of large amounts of isovalerylcarnitine. Carnitine acts by removing (detoxifying) intramitochondrial isovaleryl groups and, in the presence of glycine, it promotes the formation of isovalerylglycine. We believe L-carnitine supplementation is of value in the treatment of isovaleric acidaemia and that, in the present case, L-carnitine together with a moderate dietary restriction has proved to be the optimum form of therapy.

COMMENT:

The report, a single case study, documents similar beneficial effects of therapy with L-carnitine alone to those as described in 3.1, including more efficient glycine conjugation on carnitine supplementation. The authors, however, proceeded to demonstrate that high plasma glycine levels can cause neurotoxicity with previously used dosage regimens, and that in their patient, therapy with L-carnitine in the presence of normal plasma glycine levels obviated the need for supplementary glycine.

3.3 Berry GT, Yudkoff M, Segal S. Isovaleric acidemia: medical and neurodevelopmental effects of long-term therapy. J. Pediatr. 1988; 113: 58–64.

Authors' abstract: Nine patients with isovaleric acidemia were treated with a low-protein diet and supplemental glycine for up to 10 years. Carnitine was added to the therapy in four patients. Overall, the treatment was well tolerated, resulting in no significant side effects other than persistent hyperglycinemia. Normal growth was observed in all patients. Of four patients with the chronic phenotype, three, whose treatment was delayed beyond the first year of life, are mentally retarded. Two of five patients with the acute phenotype are retarded. The outcome in these two was complicated in one by neonatal intraventricular hamorrhage and in the other by therapeutic noncompliance. In our patients, only those who were treated successfully from early infancy and had no complications did not develop mental retardation. After initiation of therapy, there was a significant decrease in ketoacidotic attacks requiring hospitalization. Glycine is indicated for the treatment of acute ketoacidosis in these patients; none of the catastrophically ill newborn who received glycine died. The aim of treatment is to reduce the isovaleric acid burden to a minimum. Therapy consisting of leucine restriction with supplemental glycine and carnitine should be started as soon as possible after birth.

COMMENT:

L-carnitine supplements were well tolerated in the 4 patients for up to 4 years in this study. Glycine therapy reduced, but did not eliminate episodes of metabolic decompensation. The addition of L-carnitine supplements to established treatment with a low-protein diet and glycine, did not seem to alter the frequency of these episodes (0.3 ± 0.13 per year, n=4 with L-carnitine, 0.46 ± 0.28 without carnitine). The role of L-carnitine in isovaleric acidaemia seems therefore to be to reduce the need for glycine supplements, to allow a more liberal low-protein diet for equivalent metabolic control, and to be a safe and easily administered intravenous therapy for acute metabolic decompensation.

3.4 Mayatepek E. Kurczynski TW, Hoppel CL. Long-term L-carnitine treatment in isovaleric acidaemia. Pediatr. Neurol. 1991; 7: 137–140.

Authors' abstract: A 5-year-old girl with isovaleric acidemia was treated with long-term L-carnitine and no supplemental glycine. Clinical and laboratory data are presented. Following diagnosis and treatment at age 2 years, the frequency of acute exacerbations of metabolic acidosis was reduced and she resumed normal growth and development. L-carnitine supplementation and protein restriction may be sufficient for effective therapy of isovaleric acidemia.

COMMENT:

This study demonstrates that isovaleric acidaemia can be effectively treated long-term by L-carnitine supplementation in combination with a low-protein diet, without glycine supplements.

4. 3-Methylcrotonylglycinuria

3-Methylcrotonyl CoA, formed by dehydrogenation of isovaleryl CoA in the metabolism of L-leucine, is carboxylated by the specific D-biotin-dependent 3-methylcrotonyl CoA carboxylase to form 3-methylglutaconyl CoA. Deficient activity of the carboxylase apoenzyme causes isolated 3-methylcrotonylglycinuria with 3-hydroxyisovaleric aciduria. Secondary deficiency of this enzyme may also occur due to deficient activity of holocarboxylase synthetase, the enzyme responsible for attachment of the D-biotin to the apocarboxylase, and by deficient activity of biotinidase, responsible for release of free biotin from the lysyl residues (biocytin) arising from holocarboxylase turnover and from intestinal absorption from the diet and gut bacterial turnover. Several patients have been described, presenting in infancy variously with vomiting, diarrhoea, hypotonia, and life-threatening hypoglycaemia, ketoacidosis, hyperammonaemia and coma with micro- and macro-vesicular fatty infiltration of the liver that may be secondary to the severe carnitine deficiency that is observed. The disorder may be precipitated by mild infections and may be lethal, although asymptomatic affected siblings of patients have been observed. The disorder is characterised biochemically by the consistent urinary excretion of greatly increased amounts of 3-hydroxyisovalerate and 3-methylcrotonylglycine in the absence of other metabolites associated with multicarboxylase deficiencies. Patients do not respond *in vivo* or *in vitro* to D-biotin therapy and treatment is by moderate protein restriction and essential L-carnitine supplementation and the prognosis of treated patients appears good.

4.1 Rutledge SL, Berry GT, Stanley CA, Van Hove JLK, Millington D. Glycine and L-carnitine therapy in 3-methylcrotonyl-CoA carboxylase deficiency. J. Inher. Metab. Dis. 1995; 18: 299–305.

Authors' abstract: Genetic deficiency of 3-methylcrotonyl-CoA carboxylase (3-MCC) is a rare inborn error of leucine metabolism producing an organic acidaemia. With accumulation of 3-methylcrotonyl-CoA, there is increased production of 3-hydroxyisovaleric acid, the glycine conjugate (3-methylcrotonylglycine), and the carnitine conjugate (3-hydroxyisovalerylcarnitine). These conjugates represent endogenous detoxification products. We studied excretion rates of these conjugates at baseline and with glycine and carnitine therapy in an 8-year-old girl with 3-MCC deficiency. Her preadmission diet was continued. Plasma and urine samples were obtained after 24h of each of the following: L-carnitine 100 mg/kg per day and glycine 100, 175 and 250 mg/kg per day. Plasma and urinary carnitine levels were reduced by 80% and 50%, respectively with abnormal urinary excretion patterns. These normalized with carnitine therapy. Acylcarnitine excretion increased with carnitine therapy. The glycine conjugate, 3-methyl-crotonylglycine (3-MCG), was the major metabolite excreted at all times and its excretion increased with glycine therapy. Clearly, in 3-MCC deficiency the available glycine and carnitine pools are not sufficient to meet the potential for conjugation of accumulated metabolites, suggesting a possible therapeutic role for glycine and carnitine therapy in this disorder.

COMMENT:

This condition involves a defect of the metabolic step immediately after that which is defective in isovaleric acidaemia. Secondary carnitine deficiency is much more severe than in isovaleric acidaemia, and this may explain the hypoglycaemia and fatty infiltration found in this condition and not as a rule, in isovaleric acidaemia. This report documents the efficacy of combining glycine and carnitine therapy to maximise metabolite excretion, but minimising any increase in plasma glycine. No attempt was made to compare metabolite excretion on equimolar doses of carnitine and glycine (glycine doses 2–5 fold higher than carnitine) and interestingly, there appeared to be an effective upper dose limit for glycine, increases above which did not produce increases in metabolite excretion – a similar finding also reported in 3.2.

5. Glutaric aciduria

Glutaryl CoA occurs as an intermediate in the catabolism of L-lysine, L-tryptophan and hydroxylysine, being subsequently dehydrogenated in the mitochondria via a FAD-linked enzyme to glutaconyl CoA followed by decarboxylation on the same enzyme protein to crotonyl CoA and thence by β-oxidation finally to yield acetyl CoA. Deficiency in the activity of glutaryl CoA dehydrogenase leads to glutaric aciduria ("Type 1"). [Note: This disorder has been designated in the literature as glutaric aciduria "type 1" because glutaric aciduria also occurs in multiple acyl CoA dehydrogenase deficiency (MADD, formerly designated as "glutaric aciduria type 2").]

Patients with glutaric aciduria are characterised by severe progressive neurological deterioration, generalised dystonic cerebral palsy and choreoathetosis. Macrocephaly is generally present. Convulsions and ketoacidotic episodes may occur with death resulting associated with some similarities to Reye's syndrome. The patients appear to be of normal intelligence although this may be difficult to assess because of the severely dysarthric speech and severe impairment of motor function that leads to almost total helplessness. The disorder is generally associated with greatly increased excretion of glutaric acid (to more than 20,000 mmol/mol creatinine) and of 3-hydroxyglutaric acid (to more than 500 mmol/mol creatinine) and increased concentrations of glutaconic acid have been observed during acute episodes. Glutarylcarnitine excretion is also increased and a disproportionately profound carnitine deficiency develops. Some patients with similar clinical symptomatology have been reported to have minimal or absent abnormal organic aciduria and demonstrable glutaryl CoA dehydrogenase deficiency in cultured cells. Patients have also been reported with major organic aciduria but without clinical manifestations in the same sibships as clinically-affected individuals.

Treatment with a diet low in lysine and, in some cases in tryptophan, has been of value in reducing the excretion of glutaric acid and other metabolites. Similarly, treatment with riboflavin, the precursor to the FAD cofactor for the enzyme, will also reduce glutarate in some patients with residual enzyme activity. However, after presentation with severe neurological symptoms, little clinical benefit appears to result. Suggestion that the neurological damage may be due to defective function of the basal ganglia caused by inhibition of GABA (γ-aminobutyric acid) synthesis by glutarate and its metabolites, some patients have been treated with the GABA analogue Lioresal (Baclofen) (4-amino-4-(4-chlorophenyl)-butyric acid), with some improvement in clinical function. More recently, use of

γ-vinyl-GABA (Vigabatrin), an irreversible inhibitor of GABA transaminase, has also been tried with better success. However, the disorder is extremely severe and possibly progressive in patients following the initial neurological damage and the prognosis is generally very poor except in early detected and treated cases and the rare patient with high residual enzyme activity who responds to high doses of riboflavin and dietary restriction.

5.1 Hoffmann GF, Trefz FK, Barth PG *et al.* Glutaryl-coenzyme A dehydrogenase deficiency: A distinct encepnalopathy. Pediatrics 1991; 88: 1194–1203.

Authors' abstract: Clinical course, diagnostic and therapeutic management, and neuro-developmental outcome were evaluated in 11 patients with glutaryl-coenzyme A dehydrogenase deficiency. In 9 patients macrocephalus was present at or shortly after birth and preceded the neurological disease. In 7 children an acute illness resembling encephalitis appeared after a period of normal development; 2 had developmental delay and progressive "dystonic cerebral palsy." Later, all 9 displayed typical signs of a disorder of the basal ganglia. In 1 patient with macrocephalus the disorder was diagnosed before the onset of neurological disease; in another it was diagnosed prenatally. Computed tomography and magnetic resonance imaging scans revealed severe generalized cerebral atrophy, most striking in the frontal and temporal lobes in 10 patients. Further deterioration was halted after initiation of treatment consisting of low-protein diets, special formulas low in lysine and tryptophan, and supplements of riboflavin and L-carnitine. Only 1 patient showed a slight clinical improvement. Later, dietary therapy was discontinued in 2 older patients and relaxed in a third without observed adverse effects. Two patients in whom treatment could be initiated before the onset of neurological symptoms have developed normally. However, duration of follow-up (6 and 29 months) does not yet allow classification of glutaryl-coenzyme A dehydrogenase deficiency as a treatable disorder. Total body production of glutaric acid, reflected in the daily urinary output, was efficiently reduced by therapeutic measures. Levels of glutaric acid in plasma and cerebrospinal fluid remained unchanged, which may in part explain the overall unsatisfactory outcome. All patients presented with a severe secondary deficiency of carnitine. L-carnitine appears to play an important pathobiochemical role in the pathogenesis of the disorder. It is concluded that glutaryl-coenzyme A dehydrogenase deficiency is an acute to subacute neuropathic disorder of infancy or early childhood. Later in the course of the disease further deterioration appears to subside. The lack of significant metabolic derangements may cause this disorder to remain frequently undiagnosed. Glutaryl-coenzyme A dehydrogenase deficiency should have high priority in the differential diagnosis of any child with acute profound dyskinesia, subacute motor delay accompanied by increasingly severe choreoathetosis, and dystonia. It should especially be considered when there is macrocephalus in an infant together with progressive atrophic changes on computed tomography or nuclear magnetic resonance imaging, as neurological handicap can potentially still be prevented.

COMMENT:

This report evaluated 11 cases of glutaric aciduria Type 1 (a defect in the lysine degradation pathway). 7 patients presented with an acute encephalopathy, 2 with developmental delay and progressive athetoid cerebral palsy, 1 with hydrocephalus and 1 was the sib of an affected case. Not surprisingly, treatment did not reverse already established brain damage, but did seem to prevent further deterioration though the natural history of the condition is extremely variable. Secondary carnitine deficiency was present in most, if not all, of the patients. Treatment with L-carnitine, riboflavin (a precursor for the enzyme co-factor), and a low lysine diet was biochemically effective. Only the 2 patients treated before the onset of neurological disease did not develop developmental delay, emphasising the importance of early, presymptomatic diagnosis if brain damage is to be prevented.

DISORDERS OF FATTY ACID METABOLISM

Introduction

The β-oxidation of fatty acids provides the major part of the energy requirements of liver and muscle, particularly during fasting and catabolism. The brain does not directly β-oxidise fatty acids but utilises the ketone bodies produced by other tissues. In the β-oxidative process, long-chain fatty acids are taken up by the cell, activated by conversion into their corresponding acyl CoA esters via the action of acyl CoA synthetases, and transported into the mitochondria for β-oxidation by an L-carnitine-mediated process involving two carnitine palmitoyltransferases (CPT) and a specific carnitine-acylcarnitine translocase. L-carnitine facilitates the process by allowing transfer of the acyl moieties without loss of the high energy bond introduced on synthesis of the acyl CoA esters. Once within the mitochondria and converted back to their acyl CoA esters, the long-chain fatty acids enter the β-oxidation spiral. The acyl CoA esters are first dehydrogenated to trans-2-enoyl CoA esters via the action of chain length-specific acyl CoA dehydrogenases. Fatty acyl CoA dehydrogenases are part of a family of mitochondrial FAD-dependent acyl CoA dehydrogenases [including those involved in branched-chain amino acid metabolism (e.g. isovaleryl CoA dehydrogenase) and glutaryl CoA dehydrogenase]. The FAD cofactor associated with the dehydrogenases is reduced and passes its electrons to a common electron transfer flavoprotein (ETF) which in turn passes them to coenzyme Q in the electron transport chain via the action of ETF dehydrogenase. A defect in either ETF or ETF dehydrogenase will affect simultaneously all associated dehydrogenase systems to produce a multiple (acyl CoA) dehydrogenase deficiency (MADD) in which other ETF-dependent dehydrogenases are also affected. Following the dehydrogenation step, the enoyl CoA esters are hydrated to L-3-hydroxyacyl CoA esters that are then dehydrogenated to the corresponding 3-ketoacyl CoA esters. Finally the latter are cleaved to yield acetyl CoA and an acyl CoA ester that is chain-shortened by two carbons from the initial substrate. The whole process is repeated down to butyryl CoA (C_4) which is finally β-oxidised to yield acetoacetyl CoA. Odd-carbon number fatty acids are oxidised by the same process finally to yield propionyl CoA. The acetyl CoA produced is oxidised to CO_2 via the tricarboxylic acid cycle in muscle tissues but in liver is primarily converted into ketone bodies via obligatory conversion into 3-hydroxy-3-methylglutaryl (HMG) CoA followed by cleavage of the HMG CoA by HMG CoA lyase to yield acetoacetate and acetyl CoA. Enzymes differing in their carbon chain length specificities occur at almost every stage in the β-oxidation pathway and many inherited disorders have been characterised since about 1983, particularly medium-chain acyl CoA dehydrogenase (MCAD) deficiency.

These disorders are commonly associated with profound hypoglycaemia and failure adequately to synthesise ketone bodies (hypoketotic hypoglycaemia). A similar clinical presentation is also associated with 3-hydroxy-3-methylglutaryl CoA lyase deficiency (3-hydroxy-3-methylglutaric aciduria) in the catabolic pathway of L-leucine but where the major functional and symptom-producing defect is in ketone body synthesis.

Accumulating acyl CoA esters in patients with these dehydrogenase deficiencies are also converted into their corresponding acylcarnitine esters both for transport out of the mitochondria and within the cell and as a detoxification process for subsequent excretion into the urine. During acute episodes, L-carnitine insufficiency occurs. Systemic or primary carnitine deficiency caused by a specific inherited transport defect in muscle and kidney (and fibroblasts) but not liver, associated with hypoglycaemia and progressive hypertrophic cardiomyopathy and skeletal muscle weakness, is also known and is rapidly responsive to carnitine therapy at pharmacological doses.

6. Medium-chain acyl CoA dehydrogenase (MCAD) deficiency

Patients with medium-chain acyl CoA dehydrogenase (MCAD) deficiency may present acutely with acidosis, hyperammonaemia, profound hypo-ketotic hypoglycaemia, encephalopathy, hepatomegaly and pathologically with severe microvesicular fatty infiltration of the liver. Features may thus closely resemble those of Reye's syndrome. The age of initial presentation varies widely with some patients presenting acutely in early infancy with all the features described above, while others present in later childhood or even as adults primarily with hypoglycaemic episodes or muscular weakness, although the clinical history often reveals milder episodes preceding the more acute presentation. Patients excrete large amounts of dicarboxylic acids (C_6 – C_{10}) and unsaturated dicarboxylic acids during acute episodes in which suberic acid (C_8) and its conjugates predominate and suberylglycine (derived from the block in medium-chain dicarboxylic acid β-oxidation) is excreted as a most characteristic and diagnostic metabolite. C_8 – C_{10} fatty acids also accumulate in blood plasma and *cis*-dec-4-enoic acid has been shown to be particularly characteristic of the disorder. The enzyme deficiency leads to accumulation of medium-chain acyl CoA esters which are then converted into their corresponding acylcarnitine esters that accumulate in the blood and are excreted into the urine: the disorder is thus characterised also by increased excretion of esterified carnitine into the urine, primarily as octanoylcarnitine with smaller amounts of hexanoylcarnitine, and free carnitine concentrations may be low.

Diagnosis may not be easy since patients generally show no dicarboxylic aciduria when clinically well and careful assessment of patients with recurrent hypoglycaemia and suspected MCAD deficiency is essential. Asymptomatic patients usually excrete detectable amounts of octanoylcarnitine (increased on L-carnitine administration, 100 mg/kg) and suberylglycine and careful analysis usually can differentiate the patient with MCAD deficiency. Accumulating evidence suggests that MCAD deficiency is relatively common with more than 100 known cases although many cases probably remain undiagnosed. There is a spectrum of clinical presentations dependent upon the level of residual fatty acid oxidising ability and individual genotypic variations. Acute management of patients with MCAD deficiency is directed towards rapid correction of the profound and potentially lethal hypoglycaemia. L-carnitine may also be of value in acute episodes by promoting excretion of accumulating medium-chain

acyl moieties as acylcarnitines. Regular maintenance is relatively simple with avoidance of fasting, particularly when extended periods of exercise are possible, and use of a diet with moderate restriction of fat intake and increased carbohydrate with, particularly, a carbohydrate-containing drink or snack before bedtime. In some instances, the use of corn starch may be of value as a bedtime drink. Since acute episodes may be induced by mild infections and after immunisations, care must be taken during such events to monitor the patient and maintain blood glucose levels. Similar caveats apply during any prolonged surgical procedure and with appropriate measures, no adverse effects of surgical procedures have been noted. Prognosis of the correctly diagnosed and treated patient appears excellent.

6.1 Roe CR, Millington DS, Maltby DA, Bohan TP, Kahler SG, Chalmers RA. Diagnostic and therapeutic implications of medium-chain acylcarnitines in the medium-chain acyl-CoA dehydrogenase deficiency. Pediatr. Res. 1985; 19: 459–466.

Authors' abstract: The medium-chain acyl-CoA dehydrogenase deficiency is one of several metabolic disorders presenting clinically as Reye syndrome. Evidence is presented for a characteristic organic aciduria that distinguishes this disorder from Reye syndrome and other masqueraders characterized by dicarboxylic aciduria. The key metabolites, suberylglycine and hexanoylglycine, are excreted in high concentration only when the patients are acutely ill. More significantly, using novel techniques in mass spectrometry, the medium-chain defect is shown to be characterized by excretion of specific medium-chain acylcarnitines, mostly octanoylcarnitine, without significant excretion of a normal metabolite, acetylcarnitine, in four patients with documented enzyme deficiency. Similar studies on the urine of two patients reported with Reye-like syndromes of unidentified etiology have suggested the retrospective diagnosis of medium-chain acyl-CoA dehydrogenase deficiency. Administration of L-carnitine to medium-chain acyl-CoA dehydrogenase deficiency patients resulted in the enhanced excretion of medium-chain acylcarnitines. Octanoylcarnitine is prominent in the urine both prior to and following L-carnitine supplementation. The detection of this metabolite as liberated octanoic acid, following ion-exchange chromatographic purification and mild alkaline hydrolysis, provides a straightforward diagnostic procedure for recognition of this disorder without subjecting patients to the significant risk of fasting. In view of the carnitine deficiency and the demonstrated ability to excrete the toxic medium-chain acyl-CoA compounds as acylcarnitines, a combined therapy of reduced dietary fat and L-carnitine supplementation (25 mg/kg/6 h) has been devised and applied with positive outcome in two new cases.

COMMENT:

The organic aciduria and clinical picture that we now recognise as MCAD deficiency was first described in the mid-1970s, but it was not until 1982 that the specific enzyme defect in fatty acid β-oxidation was correctly identified. Although now some 10 years old, this paper establishes the fundamentals of MCAD deficiency: it identifies the characteristic medium-chain-length acylcarnitines in the urine of affected patients, excretion of which leads to secondary carnitine deficiency. Administration of L-carnitine increases the excretion of the toxic (by sequestration of mitochondrial CoASH; detergent properties) medium-chain-length fatty acyl groups as carnitine esters, providing the basis for both acute and chronic therapy with L-carnitine in this condition. Long-term combined therapy with low-fat diet and L-carnitine administration proved clinically efficacious in 1 patient and resulted in sustained biochemical improvement in a second.

6.2 Schmidt-Sommerfeld E, Penn D, Kerner J, Bieber LL, Rossi TM, Lebenthal E. Quantitation of urinary carnitine esters in a patient with medium-chain acyl-coenzyme A dehydrogenase deficiency: Effect of metabolic state and L-carnitine therapy. J. Pediatr. 1989; 115: 577–582.

Authors' abstract: Urinary carnitine esters were quantitated in an infant with medium-chain acylcoenzyme A dehydrogenase deficiency by means of a highly sensitive and specific radioisotopic exchange high-pressure liquid chromatography method. During fasting, the excretion of free carnitine and of acetylcarnitine, octanoylcarnitine, and hexanoylcarnitine was increased. The fractional tubular reabsorption of free carnitine was decreased, suggesting a renal leak of free carnitine. In the symptom-free, fed state, only minor amounts of free carnitine and of short-chain acylcarnitine, octanoylcarnitine, and hexanoylcarnitine were present in urine, and carnitine loss occurred in the form of "other" carnitine esters not exceeding that of control subjects. During L-carnitine therapy, the excretion of free carnitine, short-chain acylcarnitine, octanoylcarnitine, and hexanoylcarnitine, and particularly of "other" carnitine esters, was increased, suggesting a possible detoxifying effect of administered carnitine that is not confined to the elimination of octanoic and hexanoic acids. The employed method detects very low urinary concentrations of octanoylcarnitine and hexanoylcarnitine (less than 1 μmol/L) characteristic of medium-chain acyl-coenzyme A dehydrogenase deficiency and may be useful in screening for this disease, which has been associated with sudden infant death.

COMMENT:

This paper raises the possibility of a decreased fractional tubular reabsorption of free carnitine as a possible aetiology for the secondary carnitine deficiency. This based excretion on urinary creatinine as a constant which we now know not to be necessarily true. Administration of L-carnitine produced an outpouring of acylcarnitine as in 1.1. The detection of acetylcarnitine (unlike 1.1) may reflect the relatively high residual enzymic activity (15%).

6.3 Treem WR, Stanley CA, Goodman SI. Medium-chain acyl-CoA dehydrogenase deficiency: metabolic effects and therapeutic efficacy of long-term L-carnitine supplementation. J. Inher. Metab. Dis. 1989; 12: 112–119.

Authors' abstract: Medium-chain acyl-CoA dehydrogenase deficiency is a recently described inborn error of metabolism characterized by episodes of coma and hypoketotic hypoglycaemia in response to prolonged fasting. Secondary carnitine deficiency has been documented in these patients as well as the excretion in the urine of medium-chain-length acyl carnitine esters, such as octanoylcarnitine. Based on the potential toxicity of medium-chain fatty acid metabolites and the beneficial responses of patients with other inborn errors of metabolism and secondary carnitine deficiency, oral carnitine has been proposed as treatment for children with medium-chain acyl-CoA dehydrogenase deficiency. We report the results of carefully monitored fasting challenges of an infant with this deficiency both before and after 3 months of oral carnitine therapy. Carnitine supplementation failed to prevent lethargy, vomiting, hypoglycaemia and accumulation of free fatty acids in response to fasting despite normalization of plasma carnitine levels and a marked increase in urinary excretion of acyl-carnitine esters. Potentially toxic medium-chain fatty acids accumulated in the plasma in spite of therapy. Based on this study of one patient, we stress that avoidance of fasting and prompt institution of glucose supplementation in situations when oral intake is interrupted remain the mainstays of therapy for medium-chain acyl-CoA dehydrogenase deficient patients.

COMMENT:

This study investigated the metabolic benefits of long-term L-carnitine therapy in a single patient with MCAD deficiency. The metabolic studies with and without L-carnitine supplementation had different end points, and only plasma glucose was subjected to multiple measurements. The paucity of data mean that a small but significant benefit could have been missed, and the reproducibility, reliability, and specificity of length of fast tolerated as a measure of carnitine efficacy has not been established. Avoidance of fasting and glucose supplementation during intercurrent illness is only sensible, but the data do not justify a general rejection of L-carnitine therapy.

6.4 Catzeflis C, Bachmann C, Hale DE, Coates PM, Wiesmann U, Colombo JP, Joris F, Delézè G. Early diagnosis and treatment of neonatal medium-chain acyl-CoA dehydrogenase deficiency: report of two siblings. Eur. J. Paediatr. 1990; 149: 577–581.

Authors' abstract: Two siblings are reported who were symptomatic in the neonatal period. The first died suddenly at 4 days of age after regurgitating a meal. The postmortem examination showed steatosis of the liver, kidney and muscle. In the second, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency was diagnosed at 3 days of age with muscle hypotonia, vomiting, hyperammonaemia and mild acidosis. Thus disorders of fatty acid oxidation should also be considered in newborns. The biochemical work up indicates that in neonates, analysis of serum medium-chain fatty acids and of acyl and free carnitine are more likely to lead to a diagnosis than determining dicarboxylic acids alone in urine. Long-term treatment was effective and monitored by the acyl/free carnitine ratio.

COMMENT:

This paper emphasises that MCAD deficiency may present in the early neonatal period, and that the organic aciduria may be very subtle and easily missed or obscured by other metabolites including drugs. Measurement of free and acyl carnitines in both urine and plasma is therefore recommended for complete diagnosis. Long-term treatment with a low-fat/high-carbohydrate diet and supplemental carnitine was well tolerated for 32 months, and seemed successful in limiting metabolic decompensation to short-lived, and easily manageable episodes.

9.5 Marsden D. Sege-Petersen K, Nyhan WL. Roeschinger W. Sweetman L. An unusual presentation of medium-chain acyl coenzyme A dehydrogenase deficiency. Amer. J. Dis. Child. 1992; 146: 1459–1462.

Authors' abstract: OBJECTIVE–To report an atypical presentation of medium-chain acyl coenzyme A dehydrogenase deficiency in a 13-year-old girl with hyperammonemic encephalopathy and orotic aciduria meeting the accepted criteria for diagnosis of a female heterozygous for ornithine transcarbamylase deficiency. DESIGN–Case report and definitive biochemical testing. SETTING–Children's hospital and university laboratory. PARTICIPANT–One teenager. INTERVENTIONS– Diagnosis and treatment with carnitine. MEASUREMENTS/MAIN RESULTS–Assay ornithine transcarbamylase deficiency had normal results. The diagnosis was confirmed by DNA analysis, which revealed homozygosity for prevalent mutation (the adenine to guanine transition at position 985). CONCLUSIONS–Patients with a clinical diagnosis of Reye's syndrome have, in general, an inborn error of metabolism. Medium-chain acyl coenzyme A dehydrogenase deficiency and other disorders of fatty acid oxidation may present long after infancy. They may mimic the presentation of defects in the urea cycle.

COMMENT:

This report describes a case of MCAD deficiency presenting for the first time in a girl aged 13 years and possibly precipitated by diet-induced weight loss, i.e. catabolism. Secondary carnitine deficiency was present. L-carnitine therapy during the acute metabolic illness was felt to be beneficial, and its use as maintenance therapy was demonstrated to enhance medium-chain fatty acyl excretion as carnitine esters.

6.6 Rinaldo P, Schmidt-Sommerfeld E, Posca AP, Heales SJ, Woolf DA, Leonard JV. Effect of treatment with glycine and L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency. J. Pediatr. 1993; 122: 580–584.

Authors' abstract: To assess the relative contribution of glycine and carnitine conjugation pathways to total acyl-group excretion, we investigated the excretion of C_6 to C_{10} dicarboxylic acids, C_6 to C_8 acylglycines, and C_6 to C_8 acylcarnitines in five symptom-free patients with medium-chain acyl-coenzyme A dehydrogenase deficiency during sequential 1-week periods as follows: (1) no treatment, (2) oral supplementation with glycine, 250 mg/kg per day, (3) oral supplementation with L-carnitine, 100 mg/kg per day, and (4) oral supplementation with both combined. In untreated patients, acylglycines and acylcarnitines represented 60% and less than 1% of the total metabolite excretion, respectively; the average acylglycine/acylcarnitine ratio was 70:1. Oral supplementation with glycine did not alter the excretion of acylglycines or acylcarnitines. L-Carnitine supplementation increased the acylcarnitine excretion sixfold and caused a 60% reduction in acylglycine excretion ($p < 0.001$); however, even with carnitine supplementation, acylglycine excretion was still 10 times greater than that of acylcarnitines. The results suggest that glycine conjugation was the major pathway for the disposal of C_6 to C_8 acyl moieties and that oral L-carnitine supplements may inhibit glycine conjugation. The findings cast doubt on the value of long-term treatment of medium-chain acyl-coenzyme A dehydrogenase deficiency with L-carnitine.

COMMENT:

This is the major paper casting doubt on the long-term benefit of L-carnitine therapy in MCAD deficiency. As such it will be hotly contested. Like much of the work on efficacy of L-carnitine it focuses on biochemical changes, presumably because of the related problems of variability of clinical disease and objective measurement of morbidity.

Glycine conjugation is undoubtedly a major route of metabolite excretion for some specific acyl moieties (but not others), but it is not rate limiting as additional glycine did not alter total metabolite excretion. Secondary carnitine deficiency occurs despite plentiful glycine for conjugation, and its reversal brings certain biochemical improvement with some evidence of corresponding long-term benefit. The major role of carnitine is improvement of mitochondrial function, not as a net excretory pathway. The fall in glycine excretion with L-carnitine therapy could equally represent removal of toxicity from residual MCAD enzyme activity and /or excretion through acylcarnitines other than hexanoyl- and octanoylcarnitines. There are far more studies hinting at/alluding to clinical benefit from L-carnitine than doubting its efficacy. 'Clinical' experience is also generally positive.

6.7 van Hove JLK, Kahler SG, Millington DS, Roe DS, Chace DH, Heales SJ, Roe CR. Intravenous L-carnitine and acetyl-L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency and isovaleric acidemia. Pediatr. Res. 1994; 35: 96–101.

Authors' abstract: The purpose of this study was to determine whether treatment with L-carnitine or acetyl-L-carnitine enhances the turnover of lipid or branched-chain amino acid oxidation in patients with inborn errors of metabolism. Increasing i.v. doses of L-carnitine and acetyl-L-carnitine were given to one patient with medium-chain acyl-CoA dehydrogenase deficiency and to another with isovaleric acidemia. Both patients were in stable condition and receiving oral L-carnitine supplements. The excretion of carnitine and disease-specific metabolites was measured. The incorporation of L-carnitine in the intracellular pool was demonstrated using stable isotopes and mass spectrometry. Increasing doses of either i.v. L-carnitine or acetyl-L-carnitine did not stimulate the excretion of octanoylcarnitine in the patient with medium-chain acyl-CoA dehydrogenase deficiency, nor did it raise the plasma levels of either *cis*-4-decenoate or octanoylcarnitine. Similarly, increasing doses of either i.v. L-carnitine or acetyl-L-carnitine did not enhance the excretion of isovalerylcarnitine in a patient with isovaleric acidemia. The excretion of isovalerylglycine actually decreased. We conclude that there was no evidence of enhanced fatty acid beta-oxidation or enhanced branched-chain amino acid oxidation *in vivo* by the administration of high doses of L-carnitine or acetyl-L-carnitine in these two patients. Because only one individual with each disorder was studied, the data are only indicative and may not necessarily be representative of all individuals with these disorders. Definite settlement of this issue will require further studies in additional subjects.

COMMENT:

One of the concerns about L-carnitine therapy in MCAD deficiency has been that the secondary carnitine deficiency has acted as a brake, limiting the transport of longer chain fatty acids in to the mitochondrion to enter the 'blocked' β-oxidation pathway: thus, administration of L-carnitine could accelerate flux through the β-oxidation pathway and exacerbate the condition. In this study (in 1 patient) there was no increase in fatty acid oxidation in response to i.v. L-carnitine administration, thus negating this concern.

6.8 Ruitenbeek W, Poels PJE, Turnbull DM, Garavaglia B, Chalmers RA, Taylor RW, Gabreëls FJM. Rhabdomyolysis and acute encephalopathy in late onset medium chain acyl-CoA dehydrogenase deficiency. J. Neurol. Neurosurg. Psychiat. 1995; 58: 209–214.

Authors' abstract: A previously asymptomatic 30-year-old man presented with rhabdomyolysis, muscle weakness, and acute encephalopathy after strenuous exertion in the cold without adequate food intake. Serum and muscle carnitine concentrations were decreased. Urinary excretion of carnitine and glycine esters and biochemical examination of skeletal muscle and fibroblasts led to the diagnosis of medium chain acyl-CoA dehydrogenase (MCAD) deficiency. A point mutation at nucleotide position 985 of the coding region of the MCAD gene was found. The MCAD protein was synthesised at a normal rate, but was unstable. In general, patients in whom the 985 point mutation has been established show much more severe clinical symptoms and other symptoms than those seen in this patient. The relation of the 985 point mutation and the residual MCAD activity to the symptoms is not as straightforward as previously thought.

COMMENT:

This study documents first onset of symptoms in a previously healthy 30-year-old man. Therapy with L-carnitine produced a temporary response. The spectrum of presentations of MCAD deficiency continues to widen.

HYPERAMMONAEMIA

[Primary and secondary, including Reye's syndrome]

Introduction

Hyperammonaemia occurs both as a primary manifestation of defects in the urea cycle and as a secondary phenomenon in several metabolic disorders, especially the organic acidurias and in many other clinical conditions. It is associated with profound neurological and metabolic symptoms and sequelae and management of hyperammonaemia in such conditions is of great importance with rapid and effective treatment being necessary. Despite their efficacy the use of benzoate and phenylacetate are not without associated side effects. L-carnitine has proved to be particularly effective in rapidly countering the metabolic and neurological effects of acute hyperammonaemia and clearly has an important role in both the acute and chronic management of this severe metabolic disorder.

7. Urea Cycle Defects

7.1 Matsuda I, Ohtani Y, Ohyanagi K, Yamamoto S. Hyperammonemia related to carnitine metabolism with particular emphasis on ornithine transcarbamylase deficiency. Enzyme 1987; 38: 251–255.

Authors' abstract: Carnitine status was evaluated in 8 patients with partial ornithine transcarbamylase (OTC) deficiency and 19 patients with secondary carnitine deficiency, who were used as positive references. Laboratory findings indicated that all patients with OTC deficiency had secondary carnitine deficiency especially in hyperammonemic attack. After L-carnitine administration in 2 patients with OTC deficiency, the number of attacks was significantly reduced in both cases.

COMMENT:

This paper describes secondary carnitine deficiency in one hyperammonaemic syndrome, partial ornithine transcarbamylase deficiency. In two cases L-carnitine (50-120mg/kg/day) was added to the existing therapy of protein restriction and sodium benzoate. This reduced hyperammonaemic attacks from 3-4 per year to one or less for the ten months carnitine was given: blood ammonia level was also significantly reduced by L-carnitine therapy.s

7.2 Mori T, Tsuchiyama A, Nagai K, Nagao M, Oyanagi K, Tsugawa S. A case of carbamylphosphate synthase-I deficiency associated with secondary carnitine deficiency – L-carnitine treatment of CPS-I deficiency. Eur. J. Pediatr. 1990; 149: 272–274.

Authors' abstract: We describe a male infant with congenital hyperammonaemia due to partial carbamylphosphate synthetase-I (CPS-I) deficiency. At 21 days of age, he had convulsions and at 53 days of age hyperammonaemic coma. Therapy with sodium benzoate, L-arginine, essential amino acids, L-carnitine and peritoneal dialysis lowered the blood ammonia levels, and his clinical manifestations improved. The CPS-I activity in liver tissue obtained by open biopsy was about 25.6% of normal values. The serum and urine free carnitine levels in the patient decreased during the hyperammonaemic crisis and were low at 7 months of age. After oral administration of L-carnitine (10 mg/kg per day) at 7 months of age, the mean blood ammonia levels decreased significantly, accompanied by an increase in serum and urine free carnitine levels. We propose the use of L-carnitine therapy to prevent secondary carnitine deficiency in patients with CPS-I deficiency as well as ornithine transcarbamylase (OTC) deficiency.

COMMENT:

This patient study extends clinical experience of benefits of L-carnitine administration to another hyperammonaemic syndrome, partial carbamyl-phosphate synthetase deficiency. Peritoneal dialysis is a recognised treatment for hyperammonaemia due to inherited metabolic disease. While it may be argued that the carnitine deficiency described by the authors early in treatment may be partly due to carnitine loss into the peritoneal dialysate, the corollary is that L-carnitine therapy may be beneficial during this procedure, especially in infancy when we know that carnitine biosynthesis is limited.

7.3 Myatepek E, Kurczynski TW, Hoppel CL, Gunning WT. Carnitine deficiency associated with ornithine transcarbamylase deficiency. Pediatr. Neurol. 1991; 7: 196–199.

Authors' abstract: An infant with X-linked recessive ornithine transcarbamylase deficiency is described who also had severe deficiency of plasma and liver carnitine during normoammonemic periods. Treatment with L-carnitine (100 mg/kg/day) for 12 months decreased the frequency of hospitalizations for hyperammonemia, although it did not alter his neurologic status. This report demonstrates that persistent carnitine deficiency may be present in patients with ornithine transcarbamylase deficiency even when plasma ammonia is normal. Carnitine evaluation and supplementation may be important in the treatment of patients with this metabolic disorder.

COMMENT:

This paper demonstrates the clinical value of L-carnitine treatment in chronic hyperammonaemia with objective data.

8. General Reviews

8.1 O'Connor JE, Costell M. New roles of carnitine metabolism in ammonia toxicity. Adv. Exper. Med. Biol. 1990; 272: 183–195.

Authors' abstract: High levels of ammonia in blood and brain due to metabolic disorders are associated with neurological abnormalities. Although the mechanism of ammonia toxicity at the CNS level is still unknown, alterations in brain energy metabolism, in neurotransmitter function and direct effects on nervous impulse have been proposed. In most hyperammonemic conditions morphological changes in the liver and brain have been demonstrated, especially in mitochondria, endoplasmic reticulum and lysosomes, together with an accumulation of intracellular lipids. The treatment of hyperammonemias is uncertain and mostly directed to reduce the level of circulating ammonia; there is no current therapy aimed to counteract the molecular effects of ammonia. Administration of carnitine prevents acute ammonia toxicity and enhances the efficacy of ammonia elimination as urea and glutamine. In addition the cytotoxic effects of ammonia, possibly arising from lipid peroxidation, are ameliorated by carnitine. These data indicate the feasibility of utilization of carnitine in the therapy of human hyperammonemic syndromes, both for reducing the levels of ammonia and preventing its toxic effects.

COMMENT:

A useful review supporting a therapeutic role for L-carnitine in ameliorating ammonia toxicity. Hyperammonaemia is the hallmark of inherited metabolic defects of the urea cycle. Hyperammonaemia often occurs in other metabolic disorders, e.g. the organic acidurias, and the therapeutic benefit of L-carnitine in any one condition may well be multi-faceted.

3.2 Leonard JV. Urea cycle disorders. In Fernandes *et al.* [eds] Inborn Metabolic Diseases. Diagnosis and Treatment, 2nd Edition. Springer-Verlag: Berlin, 1995: pp167–176 [esp. p 175].

No author's abstract (book chapter).

COMMENT:

A succinct and easily readable text that contains much clinically useful information including tables of differential diagnoses and management algorithms. It emphasises the importance of the determination of plasma ammonium, an assay that should be available at all times wherever there are paediatric departments. L-carnitine is included in the emergency regimens for hyperammonaemia before diagnosis is known (200 mg/kg/24h). The evidence suggests that L-carnitine has the potential for a role in long-term therapy.

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to further the understanding and recognition of metabolic disorders in the newborn.