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Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy ☆

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Abstract

A patient diagnosed at 9 months with a milder form of propionic acidemia was functioning at a near normal intellectual level and a normal neurological level at age 8. After 2-week history of feeling "poorly" but functioning normally, she became acutely ill and succumbed to heart failure and ventricular fibrillation in 12 h. At post-mortem the heart was hypertrophied and had low carnitine levels, despite carnitine supplementation and repeatedly normal plasma carnitine levels. The findings in this patient provide a possible mechanism for the cardiac complications that are becoming more apparent in propionic acidemia.
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Introduction

Cardiac diseases, including heart failure and arrhythmias, have been recognized as important manifestations of disorders of energy metabolism for a number of years. Virtually all genetic abnormalities of the mitochondrial electron transport chain have been associated with cardiac disease [1]. Similarly, disorders of fatty acid oxidation are now recognized as placing patients at high risk of overt or occult heart disease, especially those conditions involving longer chain fatty acid oxidation [2].

Both systemic [3,4] and muscle carnitine deficiency [4] have been well known to be associated with cardiomyopathy and cardiac arrhythmias for nearly a generation.

More recently Massoud and Leonard [5] have recognized and reported serious and life threatening cardiac abnormalities in patients with propionic acidemia, although anecdotes of heart failure and sudden death in patients with both propionic and methylmalonic acidemia had been circulating prior to that time and have been since. With the advent of carnitine therapy for these disorders, plasma-free carnitine could be maintained in the normal range and would seem to reduce the probability of carnitine deficiency as the basis for this problem. Thus, we have been left with vague but plausible notion that one or another substance accumulating was toxic to the heart as it seems to be to the brain. This was particularly plausible in those patients who demonstrated acute cardiac problem in association with episodic acidosis [5].

We report here the development of a fatal cardiomyopathy in the absence of an acute episode of decompensation in a patient with more mild and well-compensated propionic acidemia. The surprising findings of carnitine deficiency and a respiratory chain abnormality are the impetus for publishing this experience

Clinical summary

The patient, a product of a non-consanguineous union and a normal pregnancy had an uneventful perinatal period. She was diagnosed with propionic acidemia at the age of 9 months when she became ill and comatose after a minor infection. Analysis of the organic acids by GC/MS showed the characteristic pattern of propionic acidemia, with elevated levels of propionate, propionylglycine, and methylcitrate. Following intensive medical therapy and improvement of her metabolic condition, the patient was placed on a protein-restricted diet and oral supplements of carnitine. An assay of propionyl CoA carboxylase in skin fibroblast showed 18% residual activity, confirming the results of the organic acid analysis.

During the first 2 years of life the patient had several episodes of metabolic acidosis and decompensation requiring hospitalization. After that age she had less frequent crises and appeared to thrive and develop normally. She started school at an appropriate age, was an average student, and participated in ballet dancing. Occasional visits to the emergency department were necessary for IV hydration and management of metabolic decompensation over the next several years, but overall she appeared to be mildly affected.

The patient complied poorly with the dietary restriction of protein, a fact that did not appear to result in significant metabolic derangement. She received the recommended doses of oral carnitine. Vision and hearing testing were normal. Her levels of propionic acid remained mildly elevated while her protein profile was not significantly abnormal (Table 1). The patient maintained normal plasma-free carnitine levels although the total level was high due to increased esterified carnitine (Table 1).

A psychometric evaluation was performed when the patient was 7½-years-old. She received a total IQ score of 90. Her verbal, linguistic, and academic skills were below average but in line with her verbal intelligence skills.

During her last visit to the Metabolic Clinic at 8 years of age, the routine examination of plasma amino acids showed the characteristic elevation of glycine. Although the amino acid values suggested a high protein intake, valine and isoleucine levels were relatively low (Table 1).

Three days prior to her final hospitalization following a family trip to the desert, the patient complained of abdominal pain. She developed emesis and refused to take food or fluids. After 3 days, the patient was taken to the emergency room where she was found to be in mild respiratory distress with tachypnea, grunting, nasal flaring, and intercostal retractions. Her initial electrocardiogram showed sinus tachycardia and non-specific T wave abnormality (Fig. 1). The liver was slightly enlarged with a border 2 cm below the right costal margin. Biochemical data obtained upon admission to the hospital did not suggest metabolic acidosis or hyperammonemia. The chest X-rays suggested enlargement of the heart and pulmonary congestion.

In the hospital, the patient had intermittent bouts of wide complex tachycardia. The hepatomegaly became progressively worse and the patient developed rales on both pulmonary fields. A bedside echocardiogram failed to show pericardial effusion, and although other details were lacking, there was evidence of electro-myocardial dysfunction. The patient developed ventricular fibrillation and could not be resuscitated.

A blood specimen was obtained before the patient's death to measure the propionic acid which was 9 μmol/L (reference 0–5 μmol/L).

Post-mortem examination

The autopsy revealed hepatomegaly and cardiac hypertrophy. There was bilateral pulmonary congestion. The liver weighed 1050 g (normal 778 g). The heart weighed 350 g (normal 116 g). The right ventricular wall was 0.3 cm in thickness, while the left ventricular wall

Table 1
Plasma amino acid, propionic acid, and carnitine levels

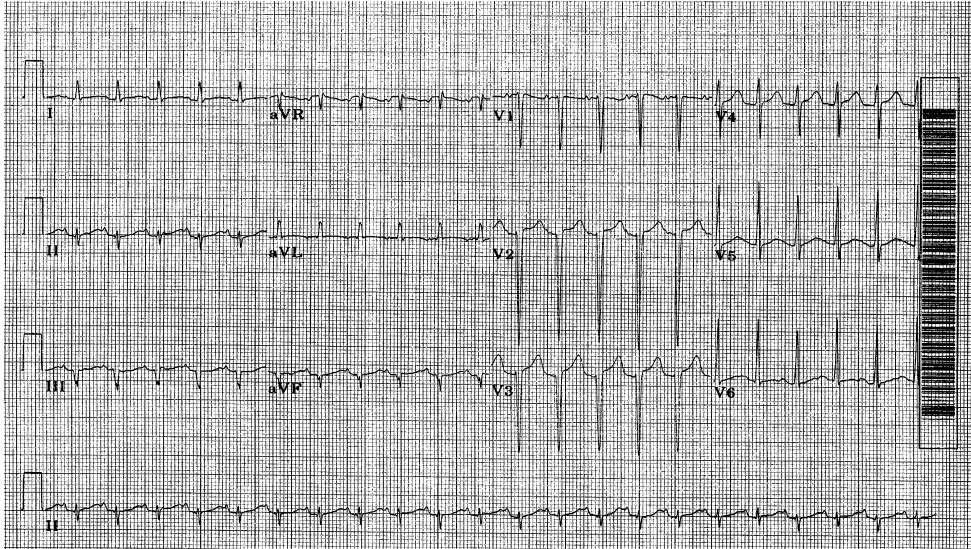|	Age (years/months)				
	6 (3/12)	6 (9/12)	7 (2/12)	7 (11/12)	8 (3/12)
Glycine (120–350 μmol/L)	907	692	1316	1045	937
Leucine (70–220 μmol/L)	73	56	52	37	294
Isoleucine (40–120 μmol/L)	18	58	27	22	50
Valine (140–350 μmol/L)	73	82	79	65	117
Propionic acid (0–5 μmol/L)	81	15	1	—	14
Total carnitine (35–80 μmol/L)	—	112	156	90	118
Free carnitine (30–70 μmol/L)	—	51	69	35	42
Acylated carnitine (5–10 μmol/L)	—	61	87	55	76

Fig. 1. Initial electrocardiogram.

was hypertrophied and 1.8 cm in thickness. The chambers were not dilated and the endocardial surfaces were smooth. There were no abnormalities of the cardiac valves. Microscopic examination of the heart showed increased myofiber size. Nuclei were enlarged and hyperchromatic. Coronary arteries were unremarkable. The pathologic examination of the brain was unremarkable. Histology gave no further insight and abnormalities were non-specific.

Biochemical analysis of carnitine on heart and skeletal muscle

Skeletal and heart muscle specimens were obtained within one hour of the patient's death. They were quenched in 2-methylbutane chilled by liquid nitrogen. Free and esterified carnitine was determined in water homogenates of muscle by spectrophotometric enzymatic assay, after extraction with methanol. Results are summarized in Table 2.

Total and free carnitine in heart muscle was very low. Low esterified carnitine content suggests a major defect in acylation/esterification pathway. Free and total carnitine in striated muscle was within normal limits but the esterification/acylation value was low.

Table 2
Carnitine content in heart and skeletal muscle

	Total carnitine	Acyl carnitine	Free carnitine
Heart muscle (9 ± 4 nmol/mg NCP)	4.5	0.2	4.3
Striated muscle (14 ± 4 nmol/mg NCP)	21	1.0	20.0

Normal values from Verity [9].

Mitochondrial oxidative enzymes assay

The activity of carnitine palmityl transferase was assayed spectrophotmetrically as described by Bieber et al. using 40 µmol/L CoA substrate derivatives. The short and long-chain acyl-CoA dehydrogenase were assayed by the reduction of 2,6-dichlorophenolindophenol (DCPIP) at 600 nm using a modification of the method of Fong and Schulz [6] in which 0.1 mmol/L KCN substituted for N-methylmaleimide. The results are shown in Table 3.

Carnitine palmityl transferase activity was normal in heart and muscle. An apparent absence of NADH cytochrome c reductase (complex I and III) was found in heart and skeletal muscle tissue. The activities of cytochrome c oxidase (complex IV) and succinate cytochrome c reductase (complex II and III) were unremarkable (Table 2).

Table 3
Enzymes of fatty acid oxidation in heart and skeletal muscle

	Heart	Muscle
Non-collagen protein (8.34 ± 2 mg/100 mg wet weight filtered)	7.4	5.6
Carnitine palmityl transferase (68–180, 108 ± 12 nmol/min/100 mg NCP)	418	141
Palmityl CoA dehydrogenase (188 ± 21 nmol/min/100 mg NCP)	99	27
Octonoyl (medium chain) (105–119, 13-42 nmol/min/100 mg NPN)	68	10
Butyryl (short chain) (161 ± 30 nmol/min/100 mg NCP)	110	59
Cytochrome c oxidase (0.9–2.8 µmol/min/100 mg NCP)	2.83	1.85
NADH cytochrome c reductase (0.4–1.1 µmol min/100 mg NCP-skeletal only)	0.0	0.0
Succinate cytochrome c reductase (0.4–0.9 µmol/min/100 mg NCP)	0.80	0.24

Analysis of long, medium and short chain acyl CoA dehydrogenase activity revealed low normal values in heart and muscle with an apparent significant deficiency of medium chain (octanoyl) acyl CoA dehydrogenase in heart and skeletal muscle.

Discussion

Cardiomyopathy as a complication of organic acidemias and particularly of propionic acidemia is now a well accepted phenomenon ([5]; internet communications). The presentation of acute decompensation in this patient was somewhat of a surprise because the patient had no obvious exacerbation of her metabolic condition. That the onset of the process occurred prior to the onset of symptoms was demonstrated by the severe cardiac hypertrophy.

Despite the severity of metabolic crises that occur in propionic acidemia, accompanied by acidosis, hyperammonemia, pancytopenia, and acute encephalopathy, cardiac dysfunction is infrequently recognized. A low index of suspicion may account for this, particularly as it may be overlooked in the face of other very severe signs and symptoms.

Carnitine deficiency has not been a prime suspect in the cardiac disease in propionic acidemia because normal plasma levels are maintained by oral supplementation. Because we have surmised that adequate levels of circulating carnitine would compensate for that lost in the esterified form in urine, we were surprised to find such reduced levels in heart, and unexpectedly not in peripheral skeletal muscle. Since hypertrophic cardiomyopathy can be caused by carnitine deficiency, it is plausible to invoke this deficiency as the cause of the cardiac abnormalities in this patient. Perhaps this mechanism is operative in other patients with this disorder who have cardiac disease, but this must be proven.

A disparity between plasma and muscle carnitine levels is not unprecedented [7]. They have shown that skeletal muscle carnitine may be low when plasma levels are maintained in the normal range. It is less clear why the carnitine uptake into the heart was impaired or why the disparity with skeletal muscle was found. What is clear is that not only were the total carnitine levels low, but the proportion of acylated carnitine was particularly low, in both muscle types.

Rather surprising and unexpected was the finding of an apparent absence of NADH cytochrome *c* reductase activity, reflecting most likely electron transport chain complex I deficiency, both in heart and skeletal muscle. The easy explanation that this was due to inadequate handling of the specimens seems diminished by normal activity of enzymes reflecting complexes II and III (succinate cytochrome *c* reductase) and complex IV (cytochrome *c* oxidase) in the same specimens.

We are not aware of a similar observation having been made in the past. The role this mitochondrial complex dysfunction could have played in the development of the cardiac complication in our patient is difficult to know without additional information. It is tempting to theorize that the resultant energy deficiency could have compromised myocardial function at a time of extremely high demand and that previous stability of the metabolic status allowed for years of proper response to other high demand situations. How this secondary complex I deficiency came about is a mystery and we are reluctant to dignify this with speculation. The primary pathologic effect of propionic acidemia has not, to our knowledge, been elucidated so that any numbers of mechanisms, including this one are possible.

We concluded from the previous observations that cardiac decompensation is a possible, and serious, complication during acute intermittent crises in patients with propionic acidemia. We have previously seen a newborn with this disorder that showed severe and reversible cardiac failure, documented on echocardiogram, but not otherwise defined. Another adolescent had recurrent palpitations and supraventricular tachycardia that is easily controlled and has not progressed in nearly a decade. Other instances of cardiac symptoms and signs and, in some cases, death have been related on a metabolic listserv (metab-l@franken.de) and at meetings in conversation. This cannot be a rare occurrence.

The factors related to the development of the dysfunction, at times of crisis, are not necessarily the same as those involved when the patient is in a more stable state.

Since mitochondrial production of energy is pivotal to adequate functioning of all body organs, it is not difficult to imagine that any of a multitude of different processes taking place in the mitochondria could cause dysfunction if not proceeding normally. Our findings point to dysfunction at different levels of the energy production chain in our patient. Recently, Gebhardt reported two patients with propionic acidemia, who had MRI changes similar to hypoxic encephalopathy, findings compatible with a mitochondrial cytopathic picture (email on metabolic listserv, Metab-l, 09/15/2004). All of these findings are consonant with Gregersen's studies [8] which found that propionate, at concentrations found in the plasma of patients with propionic acidemia, inhibits both pyruvate and 2-oxo-glutarate oxidation by 50%.

Our response to this unfortunate case and our other experiences, has been to do cardiac evaluations at least biannually in all patients with propionic acidemia, including in the examination an electrocardiogram, a rhythm strip, and an echocardiogram including ejection fraction. Thus far we have seen no abnormalities in six patients over a period of 8 years. We would respond

to any abnormality with an increased dose of carnitine, unsure, however, if this is either appropriate or helpful. Only increase vigilance and careful post-mortem studies, when we fail, will elucidate the situation further.

Acknowledgments

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