Increased C3-Carnitine in a Healthy Premature Infant

Kimberly A Chapman,1 Michael J. Bennett,2 and Neal Sondheimer3

CASE

A 5-day-old male infant with an increased dried blood spot propionylcarnitine (C3-carnitine) value of 7.93 μmol/L (cutoff <6.79 μmol/L) was identified by the New Jersey state newborn screening program. C3-carnitine is used as a screening tool for methylmalonic and propionic acidemias, potentially fatal but treatable inborn errors of metabolism. The initial screen values provided a calculated C3:C2 carnitine ratio of 0.23 (cutoff <0.32, mean 0.074) and a C3:C16 ratio of 2.51 (cutoff <4.16, mean 0.96). The child was an inpatient at an outlying neonatal intensive care unit. He was born at 35 weeks estimated gestational age, required continuous positive airway pressure for a short time after birth, and transitioned quickly to room air. He was taking regular feedings with a cow’s milk protein–based formula.

On day of life 6, the patient developed a mild acidosis (pH 7.24 on arterial blood gas testing). Because methylmalonic and propionic acidemia could not be excluded while confirmatory test results were pending, feedings were discontinued, intravenous hydration with glucose-containing fluids was initiated, and the infant was transferred to our institution. On arrival the child appeared well, was alert, and had normal growth parameters and no tachypnea. He had good tone and normal reflexes, and laboratory studies showed no acidosis. We allowed normal feedings and proceeded with the diagnostic evaluation.

DISCUSSION

Although C3-carnitine appears in the blood, the active metabolite within the mitochondrion is propionyl-CoA. Propionyl-CoA is an intermediate in the degradation of several amino acids. It can also appear as an intermediate of odd-chain fatty acid metabolism and exogenously as a derivative of propionate that is generated by gastrointestinal flora. Normally propionyl-CoA is metabolized to methylmalonyl-CoA by the action of propionyl-CoA carboxylase (PCC), but if the metabolite is in excess the propionyl species is released from the mitochondrion after conversion by carnitine palmitoyl transferase II to the corresponding acylcarnitine (Fig. 1).

The differential diagnosis for increased C3-carnitine in a newborn includes inborn errors of metabolism, vitamin B12–deficiency, and false-positive results (1). The associated inborn errors of metabolism include PCC defects that cause propionic acidemia. Children with this condition potentially have the greatest elevations in C3-carnitine because of an immediate backup of metabolic flux resulting in increased concentrations of propionyl-CoA. Defects in processing of the cofactor for PCC, biotin, could in theory lead to C3-carnitine elevation, but isolated elevations of C3-carnitine in patients with biotinidase deficiency or holocarboxylase synthetase deficiency have not been reported.

The largest group of defects associated with C3-carnitine elevation involves the downstream enzyme methylmalonyl-CoA mutase (MMM). MMM converts methylmalonyl-CoA to succinyl-CoA, an intermediate in the Krebs cycle. This enzyme is one of 2 in the body that uses vitamin B12 as a cofactor. C3-carnitine is the newborn screen metabolite used for detection of MMM deficiency (known as methylmalonic acidemia) because C4-dicarboxylcarnitine elevations (MMA-carnitine or succinyl-carnitine) are not consistently or sufficiently increased to enable differentiation of patients from those who are unaffected; the backup to propionyl-CoA and C3-carnitine is more readily detectable. A broad variety of defects in vitamin B12 processing, known as cobalaminopathies, can lead to disorders with a biochemical overlap with methylmalonic acidemia.

Maternal vitamin B12 deficiency, and vertical transmission of this deficiency, is a known cause of C3-carnitine elevation (2, 3). This defect is not isolated to the newborn period; breast-fed infants of vegan mothers with B12 deficiency have been reported with neurological impairment and methylmalonate excre-
tion (4). Commercially available formulas and term breast-milk from mothers with normal B12 metabolism have adequate concentrations of B12 to avoid such complications.

The use of diagnostic laboratory evaluations can help to differentiate the causes of C3-carnitine elevations (Table 1). The acylcarnitine profile of our patient on day of life 5 did not detect C3-acylcarnitine, nor did a repeat acylcarnitine analysis on day of life 6. There was no increased methylmalonate, the diagnostic species of methylmalonic acidemia, in urine organic acids measured by GC-MS. Homocysteine, often increased in B12 deficiency and some defects of cobalamin metabolism, was not detected. Methylcitrate and 3-hydroxy propionate, additional markers of propionic acidemia, were absent from urine organic acids, effectively ruling out defects in PCC. A serum B12 concentration obtained on day of life 5 was in the lower end of the normal range (3300 pg/L, normal reference interval 2930–12 080 pg/L).

On further review of the history, we learned that the mother was diagnosed with anemia during the pregnancy. On closer questioning, she disclosed that 3 years before the pregnancy she had undergone gastric bypass for the purpose of weight loss. She could not recall having received supplemental vitamin B12 following the procedure.

**DIAGNOSIS**
The diagnosis was vitamin B12 deficiency due to maternal vitamin B12 deficiency after gastric bypass.

![Fig. 1. Propionate metabolism.](image)

**Table 1. Diagnostic workup and expected values for newborns with elevated C3-carnitine (1).**

<table>
<thead>
<tr>
<th>True diagnosis</th>
<th>B12</th>
<th>Acylcarnitine profile</th>
<th>Urine organic acids</th>
<th>Plasma amino acids</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acidemia</td>
<td>Normal</td>
<td>↑ C3</td>
<td>↑ Methylcitrate, 3-OH propionate, propionyl glycine</td>
<td>↑ Glycine, Normal homocysteine</td>
<td>↑ Ammonia, acidosis</td>
</tr>
<tr>
<td>Methylmalonic acidemia</td>
<td>Normal</td>
<td>↑ C3</td>
<td>↑ ↑ Methylmalonate</td>
<td>Normal homocysteine</td>
<td>Severe acidosis, ↑ Ammonia</td>
</tr>
<tr>
<td>Cobalaminopathy</td>
<td>Normal</td>
<td>↑ C3</td>
<td>↑ Methylmalonate</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>B12 deficiency</td>
<td>Low</td>
<td>↑ C3</td>
<td>↑ Methylmalonate</td>
<td>+/− Elevated homocysteine</td>
<td>None</td>
</tr>
<tr>
<td>Corrected B12 deficiency</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
</tr>
</tbody>
</table>
RESOLUTION OF CASE
Vertical transmission of vitamin B$_{12}$ deficiency caused a metabolic disturbance in the child on the second day of life, at the time of newborn screening. The B12 deficiency in our patient, however, had been corrected by the time of transfer to our institution. After 5 days of enteral feeding, provision of dietary vitamin B$_{12}$ intramuscularly was required for absorption. Patients who have had gastric bypass are at significant risk for B12 deficiency because of the loss of intrinsic-factor secretion. Patients should receive 1 mg intramuscular B12 every 3 months or 500 µg intranasal B12 weekly following gastric bypass.

Newborn screening is designed to detect inborn errors of metabolism in a time course that improves prospects for treatment and survival. Newborn screening was initially developed for detection of phenylketonuria, but has expanded in most states to include other conditions such as fatty acid oxidation defects, amino acidopathies, and organic acidopathies. Increased C3-carnitine presents a diagnostic challenge because of the wide range of possible causes, including false-positive results, vitamin deficiency, and life-threatening disorders such as methylmalonic or propionic acidopathy. Severe disease was unlikely in this case, because the typical infant with methylmalonic or propionic acidopathy presents with dehydration, moderate hepatomegaly, increased ammonia, ketoacidosis, poor feeding, drowsiness, axial hypotonia, and limb hypertonia.

Detection of maternal pathology (in this case B12 deficiency) through the newborn screen is not unique to this condition. Elevations in C5-OH acylcarnitine are indicative of a variety of pathologies, including 3-methylcrotonyl-CoA carboxylase deficiency, and have frequently resulted in a diagnosis of this deficiency in the mother rather than in the newborn. The mechanism in these patients is probably distinct, with 3-methylcrotonyl-CoA carboxylase elevations due to a direct maternal transfer of the acylcarnitine species. Defects in the maternal carnitine transporter have also been detected by low concentrations of carnitine on a newborn screen.

The mechanism of B12 deficiency in the patient’s mother is also not uncommon. The number of gastric bypasses performed on women of reproductive age has increased as roux-en-Y gastric bypasses in the general population have increased from 16,000 per year in the early 1990s to 103,000 in 2003. Patients who have had gastric bypass are at significant risk for B12 deficiency because of the loss of intrinsic-factor secretion that is required for absorption. Patients should receive 1 mg intramuscular B12 every 3 months or 500 µg intranasal B12 weekly following gastric bypass.

This case illustrates an unusual mechanism leading to the elevation of a diagnostic metabolite. The patient’s benign presentation in combination with family history and laboratory studies revealed the cause before any form of treatment was instituted in the infant. In this case, the mother elected to feed her child commercial infant formula, which corrected the vitamin deficiency. Had the infant been breastfed, the deficiency would have continued and acidosis resulting from impaired MMN function could have resulted. As a result of the newborn screening program, the maternal deficiency in B12 was detected and treated before the emergence of neurological symptoms, although she had already developed anemia. This unusual route of diagnosis, although it may provoke anxiety in the clinician and family, can be considered an unexpected benefit of the newborn screening program. History, examination, and metabolic laboratory studies are sufficient to expeditiously separate cases of methylmalonic and propionic acidemia from false-positive or nutritional causes of C3-carnitine elevation on newborn screening.

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References

Commentary

Charles P. Venditti

Supplemental newborn screening using acylcarnitine ester profiling has greatly expanded the scope of detection of newborns with a wide range of fatty acid oxidation disorders and organic acidoses. Each metabolite, or group of metabolites, measured by tandem mass spectrometry in the newborn blood spot carries the potential to identify infants at risk for metabolic disease. Among these markers, a positive test for increased C3 species—propionylcarnitine—causes a characteristic and immediate reaction of initiating an emergency metabolic evaluation for the at-risk infant, with good reason. Increased propionylcarnitine in the blood is a biochemical hallmark of isolated methylmalonic acidemia, propionic academia, and disorders of intracellular cobalamin processing, all potentially lethal disorders of intermediary metabolism. However, experienced clinicians and newborn screening laboratories alike recognize that increased propionylcarnitine is not a perfect disease marker and in some large series, an infant with increased C3 species will more likely be categorized as a false positive vs affected with a metabolic disorder (1), an outcome far more desirable than the diagnosis of propionic or methylmalonic acidemia.

Although hereditary metabolic disorders comprise the most common true-positive subset within the group of babies with increased propionylcarnitine (1), another category includes infants born to mothers with diminished maternal vitamin B12 stores. Maternal B12 deficiency is recognized to produce a spectrum of symptoms in the infant—ranging from frank encephalopathy with severe metabolic derangements (2) to increased propionylcarnitine (1) with mild methylmalonic aciduria (3). The mother in the current case had undergone gastric bypass without subsequent vitamin B12 supplementation, and although her biochemical and hematological parameters were not documented in this report, she presumably was vitamin B12 deficient as was the proband when initially screened. Fortunately, other than mild prematurity, the baby was well and without biochemical abnormalities such as methylmalonic aciduria. In the end, a positive newborn screen enabled recognition of untreated maternal disease and demonstrates the unexpected societal benefit that can be derived from expanded newborn screening. One now wonders whether other infants in the false-positive category for increased C3 species might be instructing us to pay more attention to whether maternal pathology is present.

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**References**


**Commentary**

Dietrich Matern

Newborn screening is used to detect disease in the newborn. Rarely do we consider that screening may uncover disease in the mother. Only in newborn screening for infectious diseases such as HIV is the identification of disease in the mother also considered a goal (1). Furthermore, it is also generally assumed that abnormal results are indicative of an underlying genetic disease and not dietary or iatrogenic factors. As this case nicely demonstrates, newborn screening can have implications and benefits not only for the baby but also for the mother. In addition, newborn screening can also benefit others when follow-up of positive test results includes further testing of asymptomatic family members, such as in medium chain acyl-CoA dehydrogenase deficiency (2).

This case also exemplifies an issue in newborn screening that needs to be considered. Many screening programs require a second dried blood spot sample to repeat the newborn screen when the first test is abnormal. If the second sample yields normal results, then the baby is deemed healthy and the first result is overruled as a false positive. By now, it should be well known that this approach is inappropriate for several fatty acid oxidation disorders, in particular very long-chain acyl-CoA dehydrogenase deficiency (3, 4). Had the same approach been applied to this case, the maternal condition would again have escaped detection because the results of follow-up acylcarnitine profiles in the baby were already normal.

With the expansion of newborn screening and the associated increase in complexity, the American College of Medical Genetics developed follow-up guidelines (freely available at www.ACMG.net) to help the practitioner efficiently and comprehensively follow up on abnormal newborn screening results. These practice guidelines are based on the analytes that can be abnormal in newborn screening and not just the conditions officially screened for in a particular screening program. These guidelines also include suggestions to evaluate family members when indicated.

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**References**