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CASE REPORT

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Persistent hypoglycemia associated with lipid storage myopathy in a paint foal

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A 12-hours-old Paint filly was examined because of weakness and dull mentation after birth. Despite IV administered dextrose, the foal remained persistently hypoglycemic with increase in serum activity of muscle and liver enzymes. A postmortem diagnosis of lipid myopathy most similar to multiple acyl-CoA dehydrogenase deficiency (MADD) was confirmed by findings of myofiber lipid accumulation, elevated urine organic acids, and serum free acylcarnitines with respect to control foals. This report details a case of equine neonatal lipid storage myopathy with many biochemical characteristics of MADD. Lipid storage myopathies should be included as a differential diagnosis in foals with persistent weakness and hypoglycemia.

KEYWORDS
beta oxidation, horse, metabolic, weakness

1 | INTRODUCTION

A 45-kg, 12-hours-old Paint filly presented to Cornell University Equine Hospital with a history of difficulty standing, progressive weakness, and dull mentation. The foal was born at 345 days gestation, stood on its own within 1 hour of birth, but developed forelimb muscle fasciculations and become recumbent within minutes of standing. Before referral, a small amount of milk reflux from the nostrils was observed after nursing.

At admission to the hospital, the foal was recumbent, obtunded, hypothermic with a rectal temperature of 99.1°F and had a heart rate of 120 beats per minute. The respiratory rate was normal (28 breaths per minute) but increased bronchovesicular sounds were auscultated bilaterally in the midthorax to cranioventral thorax. The foal's mucous membranes were hyperemic, scleral injection was noted in both eyes, and it appeared moderately dehydrated based on abnormally dry mucous membranes of the mouth and a capillary refill time of 4 seconds. Clinical examination of the mare was normal.

The foal had neutropenia (2400 cells/μL; reference interval [RI]* 4100–9500 cells/μL) with a left shift (1000 band neutrophils/μL; [RI] 0-0 cells/μL), mild toxic changes within the neutrophils, and lymphopenia (400 cells/μL; [RI] 1000–3100 cells/μL). Fibrinogen was 300 mg/dL; ([RI] 0–200 mg/dL). The biochemistry panel identified severe hypoglycemia (blood glucose [BG] 12 mg/dL;[RI] 75–131 mg/dL), hypoglobulinemia (1.7 g/dL; [RI] 2.4-4.4 g/dL), moderately increased activity of muscle enzymes (aspartate transferase [AST] 638 U/L; [RI] 111–206 U/L; creatine kinase [CK] 4476 U/L; [RI] 165–761 U/L) and mildly increased activity of liver enzymes (glutamate dehydrogenase 35 U/L; [RI] 5.6–17.1 U/L; gamma-glutamyl transferase 65 U/L; [RI] 10–32 U/L). A venous blood gas revealed a low pH (pH 7.32; [RI] 7.38- +/−0.05) and increased lactate concentration (3.9 mmol/L; [RI] 1.2-2.4 mmol/L). An endocrine panel revealed normal ACTH and cortisol but hypoinsulinemia (3.4 μIU/mL; [RI] 3.8–15.2 μIU/mL), consistent with the foal's hypoglycemia. Whole blood cardiac troponin I concentration was within normal limits (<0.04 ng/mL). Thoracic ultrasound was suggestive of midthoracic and cranioventral pleural thickening with mild areas of

Abbreviations: GBED, glycogen branching enzyme deficiency; BG, blood glucose; BHBA, beta-hydroxy-butyric acid; ETF, electron transfer flavoprotein; ETF-QO, ETF-ubiquinone oxidoreductase; ETFDH, ETF-ubiquinone oxidoreductase mitochondrial; ETFA, electron transferase flavoprotein alpha polypeptide; ETFB, electron transferase flavoprotein subunit beta; MADD, multiple acyl-CoA dehydrogenase deficiency.

consolidation. Neonatal sepsis score was calculated to be 12 and predictive of sepsis.[1] A blood culture taken before administration of antibiotics did not grow aerobic or anaerobic organisms.

Initial medical treatment consisted of IV administration of fluids: a 1 L bolus of 5% dextrose with 0.45% NaCl (5% dextrose and 0.45% NaCL injection USP, Hospira, Lake Forest, IL) supplemented with 50 additional grams of dextrose, followed by 1L 5% dextrose/0.45% NaCl and a plasma transfusion (HighGamm, Lake Immunogenics, Ontario, NY, 20 mL/kg). Broad spectrum antibiotic treatments initiated and continued throughout hospitalization included penicillin (Penicillin G Potassium, Sandoz, Princeton, NJ, 22,000 U/kg, IV, q6h); amikacin (Amiglyde, Zoetis, Kalamazoo, MI, 20 mg/kg, IV q24h) and metronidazole (Metronidazole, Teva Pharmaceuticals, Sellersville, PA, 10 mg/kg PO q8h). Selenium (Selenium E-Se injection, Merck and Company, Madison, NJ) was administered IM at the labeled dose of 2.5 mg selenium per 100 lbs. A nasogastric feeding tube (NGT) was inserted and the foal was supplemented with a combination of mare's milk and milk replacer (Mare's Match, Land O'Lakes, Shoreview, MN) at a rate of 10% of its body weight/day divided into q2h feedings. The foal improved with these initial treatments but could only stand for brief periods (<5 minutes), after which muscle fasciculations were noted and the foal would become recumbent. Despite the initial glucose resuscitation treatment and a 12-hours IV constant rate infusion of 5% dextrose/0.45 NaCl solution, hypoglycemic persisted (12–71 mg/dL) and was difficult to resolve.

Twenty-four hours after hospitalization the foal was able to nurse the mare without aspirating milk into the trachea and nursing was allowed throughout hospitalization, along with supplemental milk feeding q2h via indwelling NGT. Blood glucose monitoring every 6 hours on day 2 revealed persistent hypoglycemia (45–68 mg/dL) requiring IV administered dextrose (5% dextrose, B Braum Medical, Inc, Irvine, CA) treatments. With each dextrose treatment, the foal would appear brighter and stronger. Hypertriglyceridemia (381 mg/dL; [RI] 14–77 mg/dL) developed without lipemia and a urine dipstick analysis revealed strong ketonuria with a urine pH of 6.5. Blood beta-hydroxybutyric acid (BHBA) was increased (0.7 mmol/L; [RI]. 0.2-0.5 mmol/L). The foal was homozygous normal on testing for the GBE1 mutation.[2]

Because of the potential inability of the foal to use glycogen stores for energy, an alternative lipid-rich diet using a combination of corn oil (15 mL), added to milk and milk replacer administered via NGT q2h and a 20% lipid solution (Lipid solution IV, Nutrilipid, Braun, Bethlehem, PA; 40 mL IV slowly q2h) was initiated on the third day. Despite of the increased caloric supplementation and continued treatment with a 5% dextrose solution (0.5 L IV bolus q4h), the foal remained hypoglycemic. Serum triglycerides were further increased (421 mg/dL); the serum became lipemic and the foal had a decreased interest in nursing. Because of the hyperlipemia, IV administered lipid supplementation was discontinued. Serum CK, although decreased from day 1, remained elevated on days 2 and 3 (1264 and 1093 U/L), suggesting continuation of muscle disease. Based on its persistent weakness, inability to stand and nurse for more than a minute and continued increase in serum AST and CK activities, a semimembranosus muscle biopsy specimen was obtained.

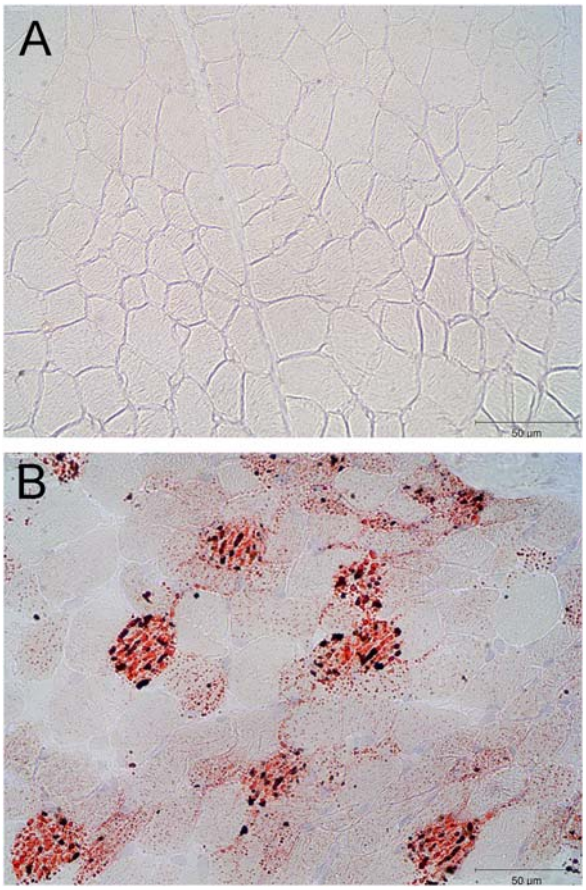

FIGURE 1 Cross-section of semimembranosus muscle from a control foal (A) and the foal with a lipid storage myopathy (B). Note the absence of staining for lipid in the control foal and the numerous large and small lipid droplets (red) in muscle fibers of the foal with lipid storage myopathy (oil red O stain)

An oral glucose absorption test (1 g/kg glucose as a 15% solution) on day 4, resulted in a >50% increase in blood glucose at 60 minutes after glucose challenge with a return to near baseline within 2 hours. The foal had now developed diarrhea, so corn oil was removed from its diet. Intravenously administered crystalloid fluids (Plasmalyte, Baxter Health Care, Deerfield, IL) with 2.5% dextrose 1L IV q6h were administered. The diarrhea improved and the foal's blood glucose, both before and after the glucose absorption test, remained in low reference range and its urine became negative for ketones. On the following day, the foal appeared brighter with a strong interest in nursing. Another attempt was made to remove dextrose supplementation, but the foal again became hypoglycemic and lethargic. Because of its inability to maintain normal blood glucose without IV administration of dextrose, combined with its recurrent weakness and lethargy, the decision was made to euthanize the foal.

Cryosections of the semimembranosus muscle biopsy collected on day 3 were stained with hematoxylin and eosin, periodic acid Schiff (PAS), amylase PAS and oil red O. Results from the semimembranosus muscle biopsy were available the day after euthanasia and indicated a lipid storage myopathy, based on numerous large densely packed lipid droplets in oil red O stains of frozen sections (Figure 1). Muscle

glycogen appeared normal on PAS stain and there was no evidence of myofiber necrosis or regeneration. Muscle fiber sizes, shapes, and distribution were within normal limits and blood vessels and connective tissue in the biopsy specimen appeared normal. To further assess a potential lipid storage myopathy, stored frozen serum that had been obtained on day 6 was submitted for assessment of carnitine and acylcarnitine concentrations, together with serum from 2 healthy, 15 and 24-days of age, foals. Urine was also submitted for assessment of urine organic acids. Serum free carnitine and acyl CoA (C2) were elevated with respect to control foals, and C3 through C18, encompassing short, medium and long chain acylcarnitines were markedly elevated with respect to foal and adult reference ranges (Table 1).[3] Urine ethylmalonic, methylsuccinic, glutaric, butyrylglycine, isovalerylglycine, 2-hydroxyglutaric, and hexanoylglycine were markedly elevated with respect to a healthy age matched (7-day-old) foal and adult control values; octanoic and decanoic acid were normal. These serum and urine findings were suggestive of multiple acyl-CoA dehydrogenase deficiency (MADD).[3–5]

Postmortem findings included myodegeneration of the tongue, semimembranosus, and diaphragm muscles, while the masseter muscles were unaffected. There was also mild diffuse hepatic lipidosis, lymphoid depletion of the spleen, and mild pyogranulomatous bronchopneumonia, consistent with aspiration pneumonia.

TABLE 1 Serum acylcarnitine profile on affected and control foals

	Affected foal	Control foal	Control foal	Reference range [3]
Age	6 days	15 days	24 days	Adult
Free carnitine	34.00	10.00	16.00	<31.1
C2	12.73	4.22	7.62	<22.50
C3	1.03	0.21	0.55	<1.47
C4	7.74	0.24	0.26	<1.09
C5	1.21	0.02	0.18	<0.47
C6	0.76	0.05	0.14	<0.11
C5-OH	0.18	0.07	0.05	<0.14
C8	0.48	0.02	0.01	<0.02
C10	1.64	0.02	0.01	<0.03
C12 : 1	0.34	0.01	0.00	<0.04
C12	1.20	0.01	0.01	<0.02
C14 : 2	0.20	0.01	0.06	<0.02
C14 : 1	0.64	0.01	0.01	<0.02
C14	0.55	0.00	0.00	<0.02
C16 : 1	0.48	0.01	0.01	<0.03
C16	0.68	0.02	0.03	<0.02
C18 : 2	0.22	0.01	0.07	<0.02
C18 : 1	0.65	0.03	0.03	<0.02
C18	0.32	0.02	0.02	<0.02

2 | DISCUSSION

This case represents a case of an equine neonatal lipid storage myopathy with many of the biochemical characteristics of MADD. The primary clinical findings in the foal were weakness, persistent hypoglycemic and biochemical evidence of both liver and muscle disease. Muscle biopsy revealed marked myofiber lipid accumulation, an unusual feature of healthy equine muscle.[3,6] Multiple acyl-CoA dehydrogenase deficiency in human neonates has a similar clinical presentation to the foal in our study, with features of muscular weakness and severe hypoglycemia within the first 24 hours of life.[5,7] Metabolic acidosis is also observed in most neonatal children with MADD but was not present in this foal. Multiple acyl-CoA dehydrogenase deficiency in human neonates is also known as glutaric acidemia type II; it is often fatal and is characterized by muscle lipidosis.[5] The initial primary rule out for the foal in our study was GBED; however, genetic testing and the lack of amylase-resistant polysaccharide in muscle biopsies made this an unlikely cause of hypoglycemia and weakness.[2,8]

A definitive diagnosis of MADD is made by identifying a specific pattern of accumulation of acylcarnitines and urine organic acids caused by aberrant fatty acid and amino acid metabolism.[4,5,7,9] Multiple acyl-CoA dehydrogenases catalyze β-oxidation of short, medium, and long chain fatty acids and metabolism of branched chain amino acids.[4,5,9] In the foal in our study, short, medium and long chain acylcarnitines were elevated in a pattern consistent with a diagnosis of MADD.[4,5,7,9] Furthermore, the foal had a pattern of increase of urinary glycine conjugates and glutaric, ethylmalonic, and methylmalonic aciduria typical of MADD.[5,7,9] Other lipid storage myopathies in humans include primary carnitine deficiency and neutral lipid storage disease, however, these were less likely because they are not characterized by abnormal acylcarnitine profiles.[5,7] Carnitine palmitoyl transferase deficiency, the most common cause of abnormal lipid metabolism in humans, as well as very long chain acyl-CoA dehydrogenase deficiency, were unlikely because they are only characterized by increases in C16-C18 acylcarnitines without urine glycine conjugates or glutaric aciduria.[7] Similarly, short and medium (C8-C10) chain acyl-CoA dehydrogenase deficiencies only have accumulation of their corresponding chain length of acylcarnitines and, in the case of short chain acyl-CoA dehydrogenase deficiency, ethylmalonic aciduria is present.[7] Thus, a primary lipid myopathy similar to MADD was most consistent with the clinical, clinical pathology, and muscle histopathology findings, as well as the serum free acylcarnitines and urine organic acids analyses in this foal.

In human neonates, MADD is most often caused by a deficiency of one of the two electron transfer flavoproteins which transfer electrons from acyl-CoA dehydrogenases to the respiratory chain: ETF (electron transfer flavoprotein, coded by *ETFA* and *ETFB*) and ETF-QO (ETF-ubiquinone oxidoreductase coded by *ETFDH*).[5,9,10] Such deficiencies are usually caused by inherited genetic mutations. Unfortunately, sequencing of these genes was not performed on the foal in the present study or its mare as the serum carnitine and acylcarnitine and urinary organic acid values were reported 3 weeks after the foal died and

samples appropriate for testing were not available. An acquired form of MADD (atypical or pasture myopathy) occurs seasonally in adult horses in North America and in Europe because of ingestion of the toxin hypoglycin A contained in the seeds of *Acer negundo* (Box elder) and *Acer pseudoplatanus* (European sycamore).[3,11–14] Seeds are usually ingested in the fall, although a lesser number of horses can be affected in the spring if they ingest shoots arising from the seeds. Metabolites of hypoglycin A block multiple acyl-CoA dehydrogenases and produce a similar pattern of increase of acylcarnitines and urine organic acids as reported in the foal in our study.[3] Horses with the acquired form of MADD typically have marked increases in blood glucose, CK, AST, and lactate, with moderate decreases in bicarbonate and variable pH.[3,15] The youngest horse documented to have acquired MADD was 4 months of age.[16] Although we believe it is unlikely that the foal in the present report had this acquired form of MADD because of the early age of onset (<12 hours old) and birth from a clinically healthy mare with no known exposure to *Acer* species trees, it cannot be ruled out. An assay for hypoglycin A was not available at the time this foal was treated.

Routine neonatal screening is used in humans to detect MADD before the onset of clinical signs and dietary treatment including riboflavin supplementation has led to prolonged survival.[17,18] Riboflavin is an obligate coenzyme of ETF.[18] There is a report of transient MADD in a newborn child caused by a riboflavin deficiency in the mother and not associated with a genetic mutation in ETF genes.[19] In one case report, breast-feeding resulted in markedly increased serum acylcarnitines, nonesterified fatty acids, and free fatty acids.[17] The best diet for the patient in that report was one with low-fat, low-protein, and high carbohydrates, a formula supplemented with corn starch as a source of slow-release carbohydrates and exogenous amylase to allow for proper carbohydrate breakdown.[17] In adults with the late-onset form of MADD, the mainstays of treatment are also dietary changes (fat and protein restriction, no fasting) and riboflavin supplementation.[20,21] Carnitine supplementation has also been found useful, as carnitine conjugates are lost in the urine and the patients become carnitine deficient.[17] It is of note that the attitude and willingness to nurse declined in the foal in our study when placed on a fat supplemented diet. In retrospect, early differentiation of a lipid myopathy versus GBED would have allowed us to provide a diet higher in carbohydrate rather than one higher in fat in an effort to provide metabolizable energy to the foal. In any event, the inability to maintain blood glucose without IV supplementation with either GBED or a lipid storage myopathy like MADD seems to be incompatible with long-term survival.

Limitations of this study include not performing biochemical testing of the clinically healthy mare and not having identically age matched control samples for serum metabolite testing. While many hormones and metabolites may change with age in growing foals, the marked differences in acylcarnitines between this foal with a lipid myopathy and our control foals were likely significant. If additional cases of neonatal lipid myopathy are diagnosed, HGA or its metabolite (MCPA) should be measured to determine if hypoglycin A toxicity is responsible. If not, genetic testing for MADD should be pursued.

*Reference normal blood values were obtained from several sources that provided normal values in 12 hours to 3 day old foals: Armengou L 2013, Hollis AR 2008, Kimura Y 2017, Pirrone A 2012, Barton M 2007 and Rossdale Laboratories 2016.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Potassium penicillin, amikacin, and metronidazole were used in a common but off-label manner; not approved for use in foals.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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