Neuromuscular disease associated with glycogen storage in a Spanish-bred filly

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METABOLIC myopathies include a group of diseases that have the common feature of accumulating normal or abnormal products of metabolism, such as glycogen and lipids. In human beings, many of these metabolic myopathies have a genetic basis (Sarnat 1983). Glycogen storage diseases are genetically determined diseases in which the normal or alternative pathways of glycogenolysis are blocked by deficiencies in the enzymatic activity needed for carbohydrate metabolism. As a result, either normal glycogen or an abnormal polysaccharide accumulates in the skeletal muscle, liver and other organs (Banker and Engel 2004). Glycogen storage diseases have been described in human beings (Cabello and others 1981), dogs (Valentine and others 2002), cats (Fyfe 1995) and birds (Tanaka and others 1996).

Two glycogen storage diseases have been described in horses: polysaccharide storage myopathy (PSSM) and glycogen branching enzyme deficiency (GBED). No enzymatic defect has been identified in horses with PSSM, making this disorder unique to the horse. PSSM has been described in Appaloosa (Valberg and others 1996, McGowan and others 2003), Morgan, Arab (Valentine and others 2000), quarter horses (Valberg and others 1992, 2001), warmblood and Andalusian horses (Quiroz-Rothe and others 2002). GBED, or glycogenosis type IV, is an autosomal recessive disorder that has only been described in quarter horses.

This short communication describes an unusual case of abnormal accumulated glycogen and sarcoplasmic masses, different from those described by Valberg and others (1992), Valentine and others (2002) and Aleman and others (2005), in a Spanish-bred filly.

A two-year-old, Spanish-bred (Andalusian) filly was presented to the University of Murcia Veterinary Teaching Hospital with a one-and-a-half-year history of chronic anaemia and eosinophilia (Table 1), decreased appetite and weakness. The filly had been regularly treated for internal parasites, and was fed a grass-alfalfa-hay mix. The referring veterinarian reported that the filly had been found positive for *Ehrlichia* species by an indirect fluorescent antibody test (IFAT), and had been treated with 7 mg/kg oxytetracycline (Panterramicina; Pfizer), administered intravenously every 24 hours for 10 days. Due to the persistent anaemia, the filly was referred to the hospital.

On physical examination at presentation, the filly had a normal rectal temperature, heart and respiratory rates, pale mucous membranes, and appeared weak, especially in the pelvic limbs. Its bodyweight (260 kg) was less than expected for its age (300 to 350 kg).

The results of haematological and serum biochemical analyses on admission are shown in Table 1. The significant laboratory findings were anaemia, lymphocytosis, hypoproteinaemia and slightly increased levels of creatine kinase (CK), aspartate transaminase (AST), alkaline phosphatase (AP), urea, creatinine, and phosphorus.

A blood culture performed at this time did not yield any growth. Coggins and Coombs tests and *Babesia* species serology yielded negative results. The IFAT was repeated and found positive at 1:800. A definitive diagnosis was made when characteristic morulae inclusions of *Anaplasma phagocytophilum* (previously *Ehrlichia equi*) were observed within granulocytes in a buffy coat. There were no abnormalities on urinalysis. Faecal analysis was moderately positive for occult blood, and gastric ulceration was observed on gastroscopy.

The filly was treated with 3 mg/kg omeprazole (Omeprazol; Guinama) and 3 g sucralfate (Sucralfato; Guinama) both administered orally, three times daily for seven days, and 3 mg/kg doxycycline (Doxycen; Cenavisa), administered intramuscularly every 12 hours for seven days, and was discharged from the hospital to recuperate on pasture.

Three months later, the red cell parameters were within reference ranges, but there was a persistent lymphocytosis (Table 1). Serum urea, creatinine, CK, AP and AST were increased (Table 1). The filly showed a decreased appetite, weakness, weight loss, muscular atrophy, fasciculations and cramps. It was treated with 20 litres of intravenous polyionic fluids (LacRinger; Braun), and three litres of 5 per cent glucose (G5; Braun) during the first 48 hours, vitamins, trace minerals and amino acids (Haemo 15; Arnolds) at a dose of
1 ml/45 kg, intramuscularly every 48 hours, and 3 mg/kg phenylbutazone (Fenilbutazona; Guinama), administered orally every 12 hours. The filly was unable to rise without assistance for several days and developed anorexia. An ELISA for equine herpesvirus type 1 (Ingezin RN; Ingenasa) and a modified direct agglutination test for equine protozoal myeloencephalitis (Antigene ToxoAD; bioMérieux) yielded negative results. A sample of the gluteus medius muscle was obtained by open biopsy under local anaesthesia with 2 per cent lidocaine (Lidocaina; Ovejero). In the final days before its death, the filly developed severe cramping and fasciculations and was recumbent. The filly died at the farm, its carcase was not available for postmortem examination.

Part of the muscle sample was fixed in 2·5 per cent glutaraldehyde in buffered cacodylate 0·1 M (pH 7·2 to 7·4) for two hours at 4°C and then embedded in Epon 812 resin according to the method of Wanson and Drochmans (1968). Semithin (1 µm) and ultrathin (50-60 nm) sections were cut with a Reichert Jung ultramicrotome (Heidelberger). The rest of the sample was embedded in tissue freezing medium (Tissue-Tek; Reichert-Jung) and rapidly frozen in liquid nitrogen-cooled isopentane (2-methyl-butane). Serial cross-sections 10 µm thick were cut in a cryostat (CM1850; Leica) at –20°C. The serial sections were stained with haematoxylin and eosin, modified Gomori's trichrome, periodic acid-Schiff (PAS) and nicotine adenine dinucleotide tetrazolium reductase (NADH-TR), as described by Dubowitz (1985). Additional cross-sections were stained for myofibrillar adenosine triphosphatase (mATPase) after alkaline preincubation (system A, described by Snow and others [1982]) and acid preincubation at pH 4·6 and 4·3 (Latorre and others 1993). Based on the mATPase reaction after preincubation at pH 4·6, the muscle fibres were classified into three types: I, IIA and IIX (Linnane and others 1999).

Histologically, most of the myofibres had structural changes within their architecture. Type IIX myofibres presented sarcoplasmic masses that were different from those described by Aleman and others (2005). The masses observed in the sample from the filly were mainly located at the centre of the myoplasm; however, some were at the periphery, close to the sarcolemma. The masses were also located in some type IIA, but not in type I, myofibres (Fig 1a). Angular atrophy was observed in all types of fibre (Fig 1a). The masses did not stain with the mATPase technique after the acid or the alkaline preincubations (Fig 1a), but stained positively with haema-

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toxylin and eosin, modified Gomori's trichome (Fig 1b), PAS (Fig 1c) and NADH-TR (Fig 2a). In addition, a small number of high oxidative myofibres presented a circular zone devoid of oxidative enzyme activity, as observed in NADH-TR-stained sections (Fig 2a). Serial sections stained with the mATPase techniques allowed identification of these myofibres as type I.

Ultrastructurally, the muscle specimen showed abundant deposits of glycogen (β glycogen in the intermyofibrillar space [Fig 2b]), surrounding the mitochondria, T-tubules, and sarcoplasmic reticulum. Deposits of glycogen were compressing but not surrounding the myofibrillar constituents. The mitochondria were usually swollen and had altered cristae (Fig 2c).

Myalgias have been rarely reported in association with ehrlichiosis in children (Schutze and Jacobs 1997), and myopathies secondary to vasculitis caused by *A phagocytophilum* infection have been reported in human beings (Van der Kolk and others 1993). However, no vasculitis was observed in the muscle specimen from this filly. To the authors' knowledge there are no scientific reports on the relationship between *A phagocytophilum* infection and myopathy in horses. Recumbency in horses has been reported in association with *A phagocytophilum* (Nolen-Walston and others 2004). Myopathies have occasionally been associated with the use of tetracyclines (mainly minocycline) in human beings (Narvaez and Vilaseca-Momplet 2004), but not in horses. The contribution of the *A phagocytophilum* infection or treatment with oxytetracycline to the filly's chronic and ongoing muscle atrophy could not be determined, but was unlikely. In addition, given the filly's age, low bodyweight, clinical signs, progressive general muscle deterioration and the results of analysis of the muscle biopsy, a congenital muscle disorder was considered.

The sarcoplasmic masses observed were present only in glycolytic myofibres (IIX and IIA). The sarcoplasmic masses were stained with haematoxylin and eosin, Gomori's trichrome and NADH-TR. PAS staining confirmed the presence of abnormal amounts of glycogen in those myofibres. Furthermore, electron microscopy revealed the accumulation of abnormal glycogen (β glycogen) surrounding the mitochondria and other organelles. Glycogen storage diseases involve defects in the degradation of glycogen as the result of a deficiency or absence of a specific enzyme; these enzymes normally catalyse reactions that convert glycogen into glucose. The common myopathological change is the presence of vacuoles containing glycogen (Banker and Engel 2004). In the present case, the glycogenolytic-glycolytic pathways were not studied.

Aleman and others (2005) described sarcoplasmic masses in type IIB (IIX) myofibres that were not membrane-bound and contained irregularly oriented and disorganised myofibrillar components, mitochondria, dilated tubules, lipofuscin and glycogen granules. The masses were found to be a non-pathological, degenerative change that resulted from wear and tear of intensely exercised muscle.

The lack of oxidative enzymatic activity at the centre of type I myofibres, in addition to the swollen appearance of the mitochondria on electron microscopy, raised the suspicion

TABLE 1: Results of haematological and serum biochemical analysis in a two-year-old Spanish-bred filly before referral, on admission to the hospital, and three months later

Parameter	Before referral	On admission	Three months later	Reference range
RBC (x 10^2/l)	6·3	5·5	7·5	6-12
PCV (%)	25·7	24	33	32-47
Haemoglobin (g/l)	88	80	109	100-160
WBC (x 10^9/l)	10·7	9·7	12·8	6-12
Neutrophils (x 10^9/l)	2·89	2·91	2·82	2·7-6·7
Eosinophils (x 10^9/l)	2·57	0·39	0·90	0·0-0·9
Lymphocytes (x 10^9/l)	5·24	6·3	9·09	1·5-5·5
Monocytes (x 10^9/l)	–	0·10	–	0·0-0·8
Platelets (x 10^9/l)	141	171	201	100-350
Total protein (g/l)	–	56	64	58-79
Fibrinogen (g/l)	–	0·4	0·4	0·2-0·4
Glucose (mmol/l)	–	3·83	7·10	4·22-7·05
Bilirubin (μmol/l)	–	13·33	12·99	8·55-34·2
AST (iu/l)	–	582	838	226-366
AP (iu/l)	–	712	589	143-395
CK (iu/l)	–	391	1325	86-300
Urea (mmol/l)	–	19·74	18·42	8·56-17·85
Creatinine (μmol/l)	–	211·27	218·34	61·88-176·8
Triglycerides (mg/dl)	–	40	42	4-44
Sodium (mmol/l)	–	–	137	132-146
Potassium mmol/l)	–	–	4·2	2·2-4·7
Chloride (mmol/l)	–	–	104	99-109
Calcium (mmol/l)	–	3·21	3·34	2·55-3·35
Phosphorus (mmol/l)	–	1·67	1·03	0·48-1·45

RBC Red blood cells, PCV Packed-cell volume, WBC White blood cells, AST Aspartate aminotransferase, AP Alkaline phosphatase, CK Creatine kinase

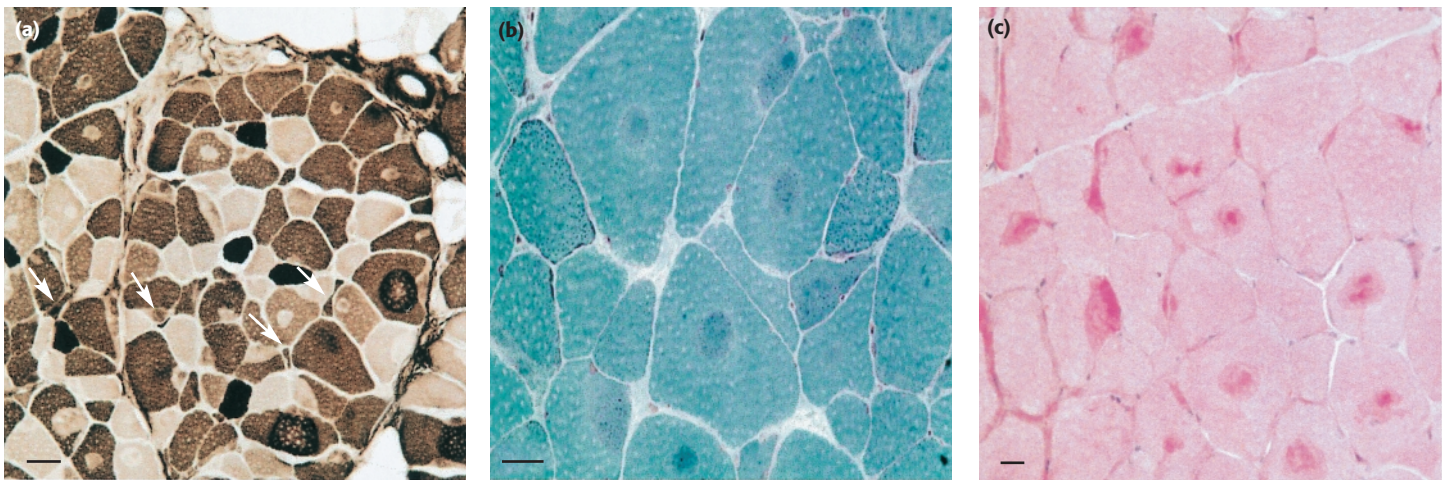

FIG 1: Histological appearance of a sample of gluteus medius muscle from a two-year-old filly. (a) Myofibrils adenine triphosphatase activity at pH 4·6, showing type I (dark), type IIA (pale) and type IIX (intermediate staining) myofibres. Sarcoplasmic masses are present in the type IIA and IIX myofibres, and there is angular atrophy in all fibre types (arrows). Bar=50 μm. (b) Sarcoplasmic masses with material, stained dark blue, within them. Modified Gomori's trichrome stain. Bar=25 μm. (c) The sarcoplasmic masses (stained dark) with internal irregular areas of darker, purple staining. Periodic acid-Schiff. Bar=20 μm

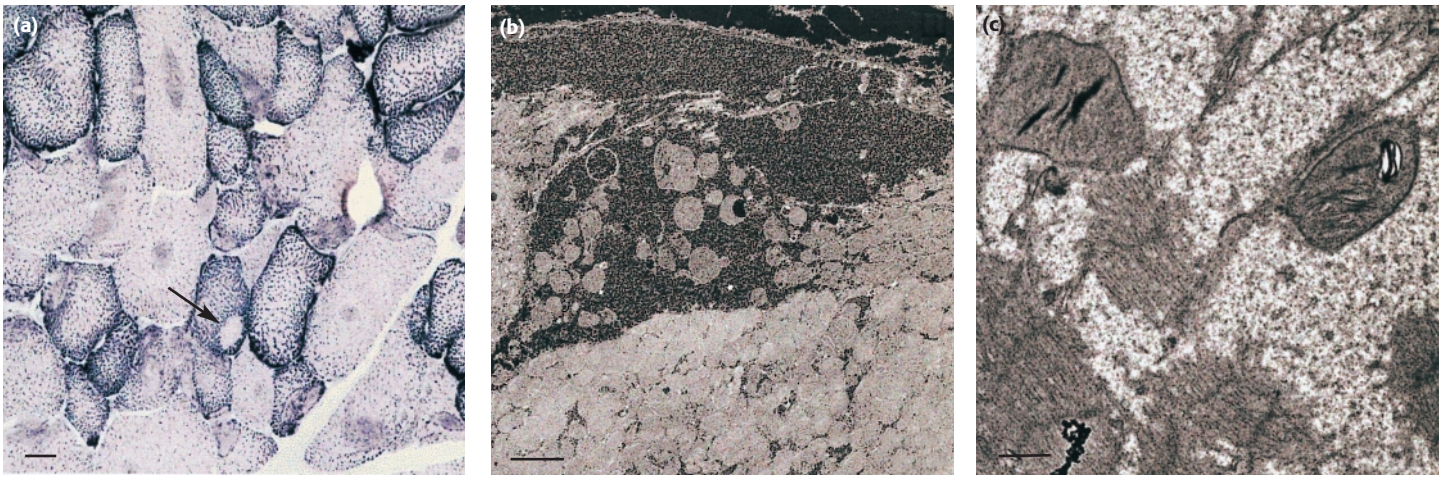

FIG 2: (a) Sarcoplasmic masses in a sample of gluteus medius muscle from a two-year-old filly, showing the presence of low oxidative myofibres. There is a lack of focal oxidative activity (arrow) in a highly oxidative fibre. Nicotine adenine dinucleotide tetrazolium reductase. Bar=25 µm. (b) Electron micrograph, showing accumulations of glycogen in the intermyofibrillar space. Bar=4·5 µm. (c) Swollen mitochondria with altered cristae; abundant deposits of glycogen are present between the mitochondria. Bar=9·45 µm

of the filly suffering from a possible mitochondrial myopathy. However, a more specific enzymatic analysis of the different mitochondrial complexes was not performed. Furthermore, blood gases, lactate and pyruvate were not measured to support a mitochondrial myopathy clinically. There is only one report in the literature of a mitochondrial myopathy due to a deficiency of the mitochondrial respiratory chain NADH-coenzyme Q (complex I), in an Arab filly with exercise intolerance (Valberg and others 1994). Central core disease has been reported in human beings with generalised weakness, although it can also be asymptomatic. This disease is characterised by markedly reduced oxidative activity in a core area, usually at the centre of type I myofibres (Carpenter and Karpati 2001). The core tends to be separated from the rest of the fibre by a slim ring of increased oxidative activity and lack of PAS staining; neither feature was observed in the filly.

Denervation atrophy results in severe (angular or group atrophy) myofibre atrophy of both type I and type II myofibres. However, in the filly only small numbers of type IIX, occasionally IIA, and rarely type I were atrophied and all the atrophy was more pronounced in the type IIX fibres. Unfortunately, no inter- and intrafascicular nerve branches were observed in the muscle specimen examined. Motor neuron disease in horses is characterised by neurogenic atrophy, which is more profound in type I myofibres; this was not the case in the filly. In addition, the filly's age and clinical signs, and the fact that it had access to fresh green forage, makes this disease unlikely. The atrophic fibres (type IIX) observed in the filly could have been associated with its catabolic state as a result of malnutrition.

On the basis of the histochemical and ultrastructural findings, it was concluded that this filly had a neuromuscular disorder characterised by sarcoplasmic masses with abnormal accumulations of glycogen different from those described by Aleman and other (2005), in addition to the abnormal findings observed in the highly oxidative myofibres (type I). There are no previous reports of this condition in horses in Spain.

In the present case, the lack of owner compliance, financial constraints and the limited availability of diagnostic tools precluded further investigation. Further diagnostic investigation and a postmortem examination would have been of great value to rule out other causes for the filly's clinical signs, and to study their association with the observed histochemical and ultrastructural features of the muscle.

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