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Equine biochemical multiple acyl-CoA dehydrogenase deficiency (MADD) as a cause of rhabdomyolysis

C.M. Westermann [a], M.G.M. de Sain-van der Velden [b], J.H. van der Kolk [a,*], R. Berger [b], I.D. Wijnberg [a], J.P. Koeman [c], R.J.A. Wanders [d], J.A. Lenstra [e], N. Testerink [f], A.B. Vaandrager [f], C. Vianey-Saban [g], C. Acquaviva-Bourdain [g], L. Dorland [b]

[a] *Department of Equine Sciences, Medicine Section, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 112, P.O. Box 80.152, 3508 TD Utrecht, The Netherlands*
[b] *Department of Metabolic and Endocrine Diseases, UMC Utrecht, Utrecht, The Netherlands*
[c] *Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[d] *Department of Genetical Metabolic Diseases, AMC Amsterdam, Amsterdam, The Netherlands*
[e] *Department of Equine Sciences, Genetic Section, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[f] *Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[g] *Service de Biochimie Pédiatrique, Hôpital Debrousse, Lyon, France*

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Abstract

Two horses (a 7-year-old Groninger warmblood gelding and a six-month-old Trakehner mare) with pathologically confirmed rhabdomyolysis were diagnosed as suffering from multiple acyl-CoA dehydrogenase deficiency (MADD). This disorder has not been recognised in animals before. Clinical signs of both horses were a stiff, insecure gait, myoglobinuria, and finally recumbency. Urine, plasma, and muscle tissues were investigated. Analysis of plasma showed hyperglycemia, lactic acidemia, increased activity of muscle enzymes (ASAT, LDH, CK), and impaired kidney function (increased urea and creatinine). The most remarkable findings of organic acids in urine of both horses were increased lactic acid, ethylmalonic acid (EMA), 2-methylsuccinic acid, butyrylglycine (iso)valerylglycine, and hexanoylglycine. EMA was also increased in plasma of both animals. Furthermore, the profile of acylcarnitines in plasma from both animals showed a substantial elevation of C4-, C5-, C6-, C8-, and C5-DC-carnitine. Concentrations of acylcarnitines in urine of both animals revealed increased excretions of C2-, C3-, C4-, C5-, C6-, C5-OH-, C8-, C10:1-, C10-, and C5-DC-carnitine. In addition, concentrations of free carnitine were also increased. Quantitative biochemical measurement of enzyme activities in muscle tissue showed deficiencies of short-chain acyl-CoA dehydrogenase (SCAD), medium-chain acyl-CoA dehydrogenase (MCAD), and isovaleryl-CoA dehydrogenase (IVD) also indicating MADD. Histology revealed extensive rhabdomyolysis with microvesicular lipidosis predominantly in type 1 muscle fibers and mitochondrial damage. However, the ETF and ETF-QO activities were within normal limits indicating the metabolic disorder to be acquired rather than inherited. To our knowledge, these are the first cases of biochemical MADD reported in equine medicine.

Keywords: Horse; Rhabdomyolysis; Myopathy; MADD; ETF; ETF-QO

Introduction

Muscle disorders are a common cause of suboptimal performance or even disability to perform. In comparison to human medicine, the etiology of muscle disorders in equine medicine is less explored. In addition to some

* Corresponding author. Fax: +31 302537970.
E-mail address: J.H.vanderKolk@vet.uu.nl (J.H. van der Kolk).

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glycogen storage diseases [1–7] an equine mitochondrial myopathy, NADH CoQ[1] reductase deficiency [8], several metabolic myopathies due to primary disorders involving ion channels and electrolyte flux and some secondary or acquired metabolic myopathies [9,10] have been observed.

Multiple acyl-CoA dehydrogenase deficiency (MADD) (also known as glutaric acidemia type II (GA-II)) (McKusick 231680) is a severe inborn error of metabolism, which can lead to early death in human patients. This autosomal recessive disease, first reported in 1976 by Przyrembel et al. is associated with a deficiency of several mitochondrial dehydrogenases that utilize flavin adenine dinucleotide (FAD) as cofactor [11,12]. These include the acyl-CoA dehydrogenases of fatty acid β-oxidation and enzymes that degrade the CoA-esters of glutaric acid, isovaleric acid, 2-methylbutyric acid, isobutyric acid, and sarcosine (a precursor of glycine). During these dehydrogenation reactions, reduced FAD donates its electrons to the oxidized form of electron transfer flavoprotein (ETF), then to ETF-ubiquinone oxidoreductase [ETF-QO, also known as ETF dehydrogenase (ETFDH)] and finally to the respiratory chain in order to produce ATP. The reduced form of ETF is recycled to oxidized ETF by the action of ETF-QO. Since electrons from FAD are transferred to ETF, deficiency of ETF or ETF-QO results in decreased activity of many FAD-dependent dehydrogenases and the combined metabolic derangements similar to those observed in MADD [13]. Heterogeneous clinical syndromes of human ETF- and ETF-QO deficiency have been described. These clinical features fall into three classes: a neonatal-onset form with congenital anomalies (type I), a neonatal-onset form without congenital anomalies (type II), and a late-onset form (type III). The latter form is also called 'ethylmalonic-adipic aciduria' or 'late-onset glutaric aciduria type II' [14,15]. The neonatal-onset forms are usually fatal and are characterized by severe nonketotic hypoglycemia, metabolic acidosis, multisystem involvement, and excretion of large amounts of fatty acid- and amino acid-derived metabolites. Symptoms and age at presentation of late-onset MADD are highly variable and characterized by recurrent episodes of lethargy, vomiting, hypoketotic hypoglycemia, strong 'sweaty feet' odour, hyperammonemia, metabolic acidosis, and hepatomegaly often preceded by metabolic stress [14,16,17]. Muscle involvement in the form of pain, weakness, and lipid storage myopathy also occurs. The organic aciduria is less clear in the milder or episodic forms of the disease. Some only manifest increased excretions of EMA and adipic acid [18]. In others, abnormal organic acid profiles are only found during periods of illness or catabolic stress.

It has been shown that riboflavin treatment and therefore elevation of FAD may alleviate the enzymatic and biochemical phenotype as well as the clinical symptoms in late-onset riboflavin-responsive MADD [19–22].

Diagnosis of human MADD is based on medical and family history, clinical examination, a characteristic organic aciduria [11,23], histopathologic abnormalities (increased lipid deposition in myofibers) as well as enzymatic and molecular characterization [16].

Several experiments have been carried out in order to obtain mice or rats with MADD like diseases. Although riboflavin-deficient rats mimicking the human disorder of MADD have been described, there are no reports of acquired MADD yet [24]. White et al. have mapped the genes for the mouse ETF-α, ETF-β, and ETFDH, determining localization of these mouse genes to chromosomes 3, 7, and 13. However, there are no mutations that might be considered as a model of human MADD [25]. To the authors' knowledge, MADD is diagnosed in no other species than man so far.

The present study describes two horses with rhabdomyolysis due to MADD. As a consequence, this animal model might be an option for further comparative research with special reference to riboflavin-responsive MADD.

Materials and methods

Case reports

Case 1 (gelding)

A seven-year-old Groninger warmblood gelding was presented at the Utrecht University Equine Clinic with a history of moderate pain following exercise. An episode of myopathy had been reported previously. Upon arrival at the clinic the horse was recumbent while shaking and sweating. There was a willingness to eat, but this caused trembling and sweating too. The horse walked straddle-legged and insecure. Further symptoms were depression and preference for lateral recumbency. Shaking and sweating developed after every minor physical activity. Myoglobinuria was also observed. Clinical examination including neurological examination and rectal exploration revealed no further abnormalities. The next day the horse was able to stand up reluctantly. However, one day later recumbency became permanent. Because of the poor prognosis the horse was euthanized at the owner's request.

Case 2 (foal)

A six-month-old Trakehner mare was sent to the Utrecht University Equine Clinic with a suspicion of colic. On arrival, the foal had a stiff gait and extremely firm gluteal, quadriceps, longissimus, and triceps muscles. She became recumbent shortly after arrival. Rectal temperature was 36.0 °C and the heart rate elevated to 56 beats per minute. The foal was dehydrated. No abnormalities were found in the digestive and neurological systems. Myoglobinuria was also observed. A tentative diagnosis of rhabdomyolysis was made. After a small improvement in the evening the condition of the foal deteriorated during the following hours. Due to the poor prognosis the horse was euthanized at the owner's request too.

[1] *Abbreviations used:* SCHAD, short-chain hydroxy acyl-CoA dehydrogenase; MADD, multiple acyl-CoA dehydrogenase deficiency; SCAD, short-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; IVD, isovaleryl-CoA dehydrogenase; ETF-α, electron transfer flavoprotein-α; ETF-β, electron transfer flavoprotein-β; ETF-QO, electron transfer flavoprotein ubiquinone oxidoreductase; ETFDH, electron transfer flavoprotein dehydrogenase; GA-II, glutaric acidemia type II; NADH CoQ, nicotinamide adenine dinucleotide coenzyme Q; FAD, flavin adenine dinucleotide; ASAT, aspartate aminotransferase; CK, creatinine phosphokinase; LDH, lactate dehydrogenase.

Blood biochemistry

Biochemical analysis of blood from the patients was performed using a CBC Analyzer from Sysmex Inc. (white blood cell count), a Synchron CX5 from Beckman Coulter Inc (concentrations of urea, creatinine and glucose as well as activities of ASAT, CK, LDH), and an ABL-605 Radiometer from Radiometer Copenhagen (pH, pCO_2, BE, HCO_3^-, and lactate). Results were compared with the laboratory's validated reference values for horses.

Analysis of organic acids and acylglycines

Identification-analyses of organic acids and glycine conjugates were carried out by gas chromatography–mass spectrometry (GC–MS) on a Hewlett Packard 5890 series II gas chromatograph linked to a HP 5989B MS-engine mass spectrometer. Prior to this GC–MS analysis, the organic acids and glycine conjugates were trimethylsilylated with *N*, *N*-bis(trimethylsilyl)trifluoracetamide/pyridine/trimethylchlorosilane (5:1:0.05 v/v/v) at 60 °C for 30 min. The gas chromatographic separation was performed on a 25 m × 0.25 mm capillary CP Sil 19CB column (film thickness 0.19 mm) from Arian/Chrompack, Middelburg, The Netherlands. The results were compared with values from 12 healthy control horses.

Analysis of free and acylcarnitines

Free and acylcarnitines in plasma and urine were analyzed as their butyl ester derivatives by electrospray tandem mass spectrometry (ESI-MS–MS) on a Micromass Quattro Ultima system equipped with an Alliance HPLC system. Results were compared with values from 12 healthy control horses. Acylcarnitine concentrations in muscle tissue were measured as described previously [26].

Muscle enzyme activities

Measurement of muscle enzyme activities was performed in lateral vastus muscle tissue of the patients, collected immediately after euthanasia in liquid nitrogen and stored at −80 °C. Lateral vastus muscle tissue of two healthy control horses was used for control measurements. The activities of medium-chain acyl-CoA dehydrogenase (MCAD), short-chain acyl-CoA dehydrogenase (SCAD) and isovaleryl-CoA dehydrogenase (IVD) were measured according to methods, which are based on the use of the substrate phenylpropionyl-CoA (for MCAD), butyryl-CoA (for SCAD) and isovaleryl-CoA (for IVD). Shortly, incubations were performed at 25 °C in a buffered medium, containing an aliquot of the muscle homogenate plus ferricenium hexafluorophosphate as the electron acceptor. After termination of the reactions by acidification, the acidified samples were centrifuged and the protein-free supernatants neutralized, followed by HPLC-analysis to separate the different acyl-CoA esters. The activity of short-chain 3-hydroxy-acyl-CoA dehydrogenase (SCHAD) was measured as described previously [27].

Assessment of muscle ETF(-QO) activities

ETF activity was measured with reduction of the artificial electron acceptor (dichlorophenol indophenol) using octanoyl-CoA as substrate and purified MCAD on the 100,000 g supernatant of sonicated muscle tissue according to a previously published protocol [28].

ETF-QO activity was revealed from homogenized muscle membranes. ETF-QO activity was measured in the reverse reaction, using succinate as substrate under anaerobic conditions at pH 8.6. Succinate dehydrogenase generates electrons which are transferred to purified oxidized ETF through Coenzyme Q and ETF-QO. Reduction of ETF was followed spectro-fluorimetrically. SCHAD was used as a control enzyme and results were compared with data from 3 healthy control horses.

Pathology

A post-mortem examination of both animals was performed macroscopically, microscopically and by electron micrography. Routine microscopical analysis of various muscles was performed using haematoxylin and eosin (H&E) stained paraffin embedded sections. In order to identify lipid droplets formaldehyde fixed specimens of *M. vastus lateralis* of the gelding were snap frozen in liquid nitrogen and cut on a Leica CM3050 microtome. Thin cryosections (10 μm) were attached to Superfrost Plus slides and incubated in 0.02 μg/μl Bodipy 493/503 (Molecular Probes, Invitrogen, Breda, The Netherlands) for 15 min in a humidified environment in order to stain neutral lipids. Lipid droplets were visualised by a Leica DMR fluorescence microscope equipped with a Photometrics Coolsnap CCD digital photo camera. Images were processed using IP-laboratory image analyses software. For muscle fiber typing a monoclonal antibody was used specific for type 1 MyHC isoform kindly provided by prof. A.F.M. Moorman, Academic Medical Centre, Amsterdam, The Netherlands.

Results

Blood biochemistry

Table 1 shows the biochemical parameters in plasma of the gelding and foal. The first day of hospitalisation revealed hyperglycemia (13.1 and 12.5 mmol/l for gelding and foal, respectively, reference range 3.9–5.6), high lactic acidemia (3.5 and 15.9 mmol/l, respectively, reference

Table 1
Biochemical parameters in plasma of gelding and foal

	Reference range	Unit	Gelding					Foal		
			Day 1	Day 2 08.00	Day 2 20.00	Day 3 08.00	Day 3 14.00	Day 1 20.00	Day 1 22.00	Day 2 08.00
pH	7.35–7.45		7.35	7.42	7.26	7.44	7.35	7.12	7.33	7.22
pCO_2	4.7–6.0	kPa	6.1	6.3	6.7	6.3	6.1	6.8	7.0	5.3
BE	−3 to +3	mmol/l	−0.4	5.8	−5.0	6.2	6.1	−13.9	0.3	−11.0
Lactate	<1.0	mmol/l	3.5	3.8	12.4		3.3	15.9	7.8	10.6
WBC	7–10	G/l		6.2		7.3		7.2	5.2	4.1
ASAT	125–275	U/l	6800			17,500		19,100		
CK	<200	U/l	180,000			237,000	240,000	100,000		
LDH	150–420	U/l	18,000					30,800		
Urea	<8	mmol/l		9.2		15.1			10.9	15.0
Creatinine	106–168	μmol/l		227					189	219
Glucose	3.9–5.6	mmol/l	13.1	6.6	9.9	8.1			12.5	

<1.0) and very high activities of CK (180,000 and 100,000 U/l, respectively, reference range ⩽200), ASAT (6800 and 19,100 U/l, respectively, reference ranges 125–275), and LDH (18,000 and 30,800 U/l, respectively, reference ranges 150–420 U/l). On the first day of hospitalisation for the foal and on the second day of hospitalisation for the gelding, a period of metabolic acidosis occurred (gelding, pH 7.26, BE −5.0 mmol/l, lactate 12.4 mmol/l and foal, pH 7.12, BE −13.9 mmol/l, lactate 15.9 mmol/l).

Analysis of organic acids, acylglycines and free and acylcarnitines

Metabolic screening of plasma and urine obtained several hours before euthanasia was performed. The most remarkable findings of organic acids in urine of gelding and foal were increased ethylmalonic acid (EMA), 2-methylsuccinic acid, lactic acid, butyrylglycine (iso)valerylglycine and hexanoylglycine (Table 2). Surprisingly, the concentration of glutaric acid in urine was normal. EMA was also increased in plasma of both animals (Table 3). Furthermore, the profile of acylcarnitines in plasma from both animals showed a substantial elevation of C4-, C5-, C6-, C8-, and C5-DC-carnitine (Table 4). Concentrations of acylcarnitines in urine of both animals revealed increased excretions of C2-, C3-, C4-, C5-, C6-, C5-OH-, C8-, C10:1-, C10-, and C5-DC-carnitine. In addition, concentrations of free carnitine were also increased.

Muscle enzyme activities

In muscle tissue of the gelding, deficiencies of three acyl-CoA dehydrogenases, namely SCAD MCAD and IVD were found. Measurement of SCHAD as a control enzyme was within normal limits. In muscle tissue of the foal, deficiencies of SCAD and MCAD were found. The activity of IVD in the foal was within normal limits (Table 5).

Table 2
Organic acids and glycine conjugates in urine

Acid or conjugate	mmol/mol creatinine		
	Gelding	Foal	Upper limit of reference range ($n = 12$)
Lactic acid	180	20,606	141
Pyruvic acid	40	483	10
3-OH-Butyric acid	17	392	133
3-OH-Isobutyric acid	13	294	138
Ethylmalonic acid	106	278	5
2-Methylsuccinic acid	47	114	5
Succinic acid	n.d.	5	12
Adipic acid	n.d.	13	4
Glutaric acid	n.d.	10	136
Butyrylglycine	++++++	++	n.d.
(iso)Valerylglycine	+	+	n.d.
Hexanoylglycine	++	+	n.d.

n.d.: not detectable.

Table 3
Organic acids in plasma

Acid	Gelding (μmol/l)	Foal (μmol/l)	Upper limit of reference range ($n = 12$) (μmol/l)
3-OH-Butyric acid	534	176	391
3-OH-Isobutyric acid	47	72	111
Glutaric acid	13	30	3
cis-4-Decenoic acid	6	20	5
3-Oxobutyric acid	10	81	13
Decanoic acid	5	82	10
Ethylmalonic acid (EMA)	38	54	1

Acylcarnitine profiling in muscle

The profile of acylcarnitines in muscle tissue of the gelding showed a substantial elevation in C4-, C5-, C6-, C8-, C10:1-, C4-DC-, and C5-DC-carnitine. Results for the foal were comparable, with even higher values for C4-, C5-, C8-, and C10:1-carnitine. In addition the free carnitine concentration in the foal muscle was remarkably reduced. Values are shown in Table 4.

Assessment of muscle ETF(-QO) activities

Measurements of ETF and ETF-QO activities as well as the activity of the control enzyme (SCHAD) in muscle biopsy of the gelding were slightly decreased compared to controls. ETF and ETF-QO activities in muscle biopsy of the foal were in the control range. In both foal and gelding the ratios (ETF/SCHAD and ETF-QO/SCHAD) were normal (Table 6).

Pathology

In the Groninger gelding as well as in the foal pathologic examination confirmed an acute rhabdomyolysis with pale, degenerated looking musculature in various muscles. Microscopically, in haematoxylin and eosin (H&E) stained paraffin sections, muscle fibers with loss of striations, floccular degeneration and myolysis were found. Some fibrotic areas were visible as were slight infiltrations with macrophages and neutrophils (Figs. 1 and 2).

Electron micrography showed subsarcolemmal accumulation of mitochondria and severe loss of mitochondrial cristae and numerous extensively damaged mitochondria (Fig. 3). Fluorescence microscopy showed microvesicular lipidosis predominantly in type 1 fibers (Fig. 4).

Discussion

This report describes for the first time biochemical MADD in equine medicine as a cause of pathologically confirmed rhabdomyolysis. The diagnosis is based on characteristic profiles of organic acids and acylcarnitines in urine and plasma. In urine, EMA and methylsuccinic acid, as well as the glycine conjugates of (iso)valerate, butyrate and hexanoate were increased as observed in human

Table 4
Relevant acylcarnitines in urine, plasma, and muscle tissue

	Urine (mmol/mol creatinine)			Plasma (μmol/l)			Muscle tissue (pmol/mg protein)			
	Gelding	Foal	Upper limit of reference range ($n = 12$)	Gelding	Foal	Upper limit of reference range ($n = 12$)	Gelding	Foal	Results of healthy controls ($n = 2$)	
Free carnitine	753	298	8	322	53	41	563	30	483	686
C2-Carnitine	458	316	1	116	12	5	1769	247	871	333
C3-Carnitine	24	30	0	4	4	1	18	101	32	9
C4-Carnitine	325	15	1	63	57	1	954	5469	112	9
C5-Carnitine	290	46	0	55	61	0	1332	9068	65	14
C6-Carnitine	40	19	0	11	9	0	126	171	11	1
C5-OH-Carnitine	2	4	0	1	1	0	20	6	2	4
C8-Carnitine	12	10	0	3	3	0	19	51	4	1
C10:1-Carnitine	2	5	0	1	2	0	10	28	1	0
C10-Carnitine	1	3	0	1	1	0	4	17	5	2
C4-DC-Carnitine	0	1	0	0	0	0	26	8	2	1
C5-DC-Carnitine	9	11	1	2	1	0	1	1	0	0

Table 5
Enzyme activities of SCAD, MCAD, IVD, and SCHAD (control enzyme) in muscle

(nmol/min/mg protein)	Gelding	Foal	Healthy control 1	Healthy control 2
Acyl-CoA dehydrogenase				
SCAD	0.24	0.32	2.27	1.72
MCAD	0.21	0.40	5.54	3.78
IVD	0.41	1.21	1.61	1.36
3-Hydroxy acyl-CoA dehydrogenase				
SCHAD	249	270	294	295

MADD patients. In contrast, 2-methylbutyric acid was not found in the urine of the horses while glutaric acid excretions were not elevated. In horses glutaric acid appears to be a normal constituent in urine in contrast to man (Table 2). We have no clear explanation for the observation that the equine patients had elevated concentrations of glutarate in plasma, but not in urine.

Acylcarnitine analyses in muscle showed increased concentrations of the short- and mid-chain carnitine esters. The strongly reduced enzyme activities of SCAD, MCAD, and IVD found in muscle tissue of the gelding supported the diagnosis MADD. The muscle tissue of the foal showed reduced enzyme activities of SCAD and MCAD while IVD appeared to be normal. This may be due to biochemical heterogeneity of the disease. This phenomenon of reduced acyl-CoA dehydrogenase activities has been described before in rats on a riboflavine-deficient diet [29].

The decreased enzyme activities suggest a defect in the electron transfer flavoprotein (ETF) system or the riboflavin synthesis system. Surprisingly, ETF and ETF-QO activities in muscle of the gelding were slightly decreased while these activities were normal in muscle of the foal. The ratios (ETF/SCHAD and ETF-QO/SCHAD) were normal for both gelding and foal, thereby excluding an (inherited) deficiency of either ETF or ETFDH. We therefore speculate that the biochemical MADD in the two horses may be caused by an exogenous factor e.g. a riboflavin deficiency or blocking, predominantly affecting SCAD, MCAD, and IVD.

When comparing clinical symptoms of both horses with those described for human MADD, the horses must have suffered from the 'late-onset' form. Similar symptoms are weakness and myopathy, though in the horses the disorder seems to be more acute and severe. Pathological investigations revealed lipid accumulation in muscle fibers and damaged mitochondria /ragged red fibers in the affected horses similar to observations in humans. Although only muscle tissue was collected immediately post mortem and available for fluorescence microscopy in order to study the presence of lipid droplets, it cannot be excluded that other organs had similar fatty changes similar to human patients. In contrast to findings in man where rhabdomyolysis is very unusual in late-onset MADD, skeletal muscle seems to be the main target organ of MADD in the equine species. However, it cannot be excluded that the enzyme deficiencies and the acute myopathy in the horses have a common cause.

Table 6
ETF, ETF-QO, and SCHAD activities (nmol/min/mg protein) in muscle

	Gelding	Foal	Healthy control 1	Healthy control 2	Healthy control 3
ETF	0.42	0.62	0.62	0.69	0.98
SCHAD	509	899	706	751	961
Activity ratio ETF/SCHAD (1000×)	0.83	0.69	0.88	0.92	1.02
ETF-QO	0.076	0.188	0.185	0.202	0.188
SCHAD	30	183	143	203	164
Activity ratio ETF-QO/SCHAD (1000×)	2.55	1.03	1.22	0.99	1.15

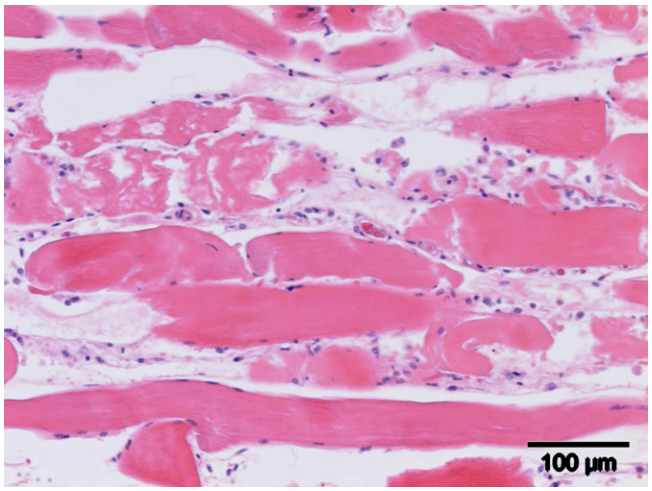

Fig. 1. Vastus lateralis muscle fibers from a seven-year-old Groninger warmblood gelding showing loss of striations, floccular degeneration and myolysis as well as slight infiltration with macrophages. At the lower section normal striated fiber (H&E stained paraffin section, objective 10×).

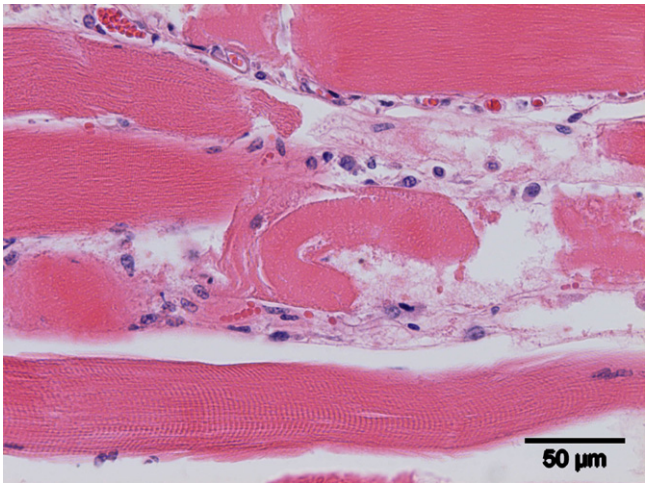

Fig. 2. Detail of Fig. 1 (H&E stained paraffin section, objective 20×).

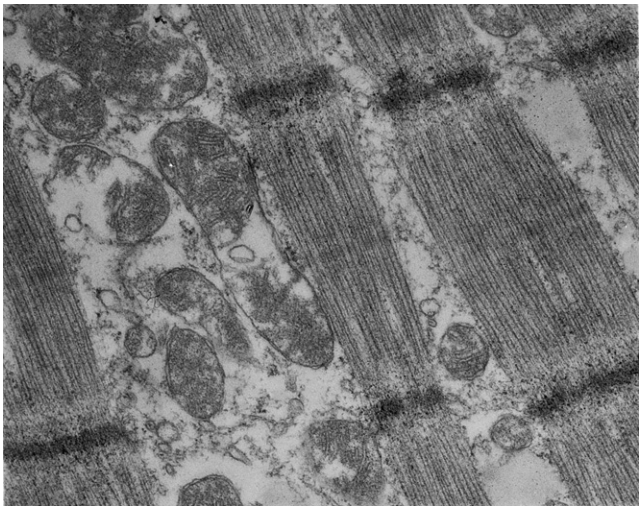

Fig. 3. Electron micrograph of lateral vastus muscle from a seven-year-old Groninger warmblood gelding illustrating severe mitochondrial damage.

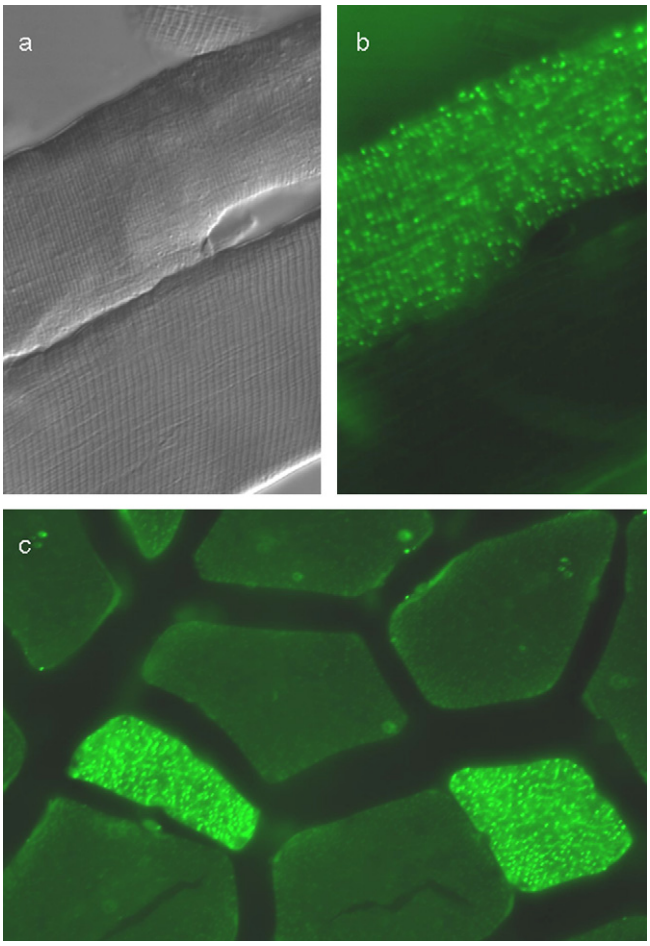

Fig. 4. Neutral lipid staining of lateral vastus muscle from a seven-year-old Groninger warmblood gelding showing microvesicular lipidosis in type 1 fibers longitudinally (b), the corresponding differential interference contrast microscopy image (a), and microvesicular lipidosis in type 1 fibers cross-sectionally (c).

Interestingly, the horses were hyperglycemic. This is in contrast with the observed hypoglycemia seen in human cases with MADD [16]. Hypoglycemia may occur as a result of increased utilization of glucose because of the block in fatty acid oxidation and the subsequent inability to synthesize ketone bodies. In addition, hepatic gluconeogenesis may be impaired. However, hypoketotic hypoglycaemia in man can also be absent. This may reflect stimulation of gluconeogenesis and ketogenesis by unimpaired oxidation of long-chain and medium-chain fatty acylCoA's [30]. We hypothesize that β-adrenergic mechanisms in stressed horses results in hyperglycemia [31]. Furthermore, ketogenesis is a very unlikely metabolic pathway in the equine species [32]. Also at odds with human MADD patients is the elevated free carnitine in the horse, which remains unexplained.

In humans, MADD is an autosomal recessive inherited disorder. Since not all patients suffering from MADD have mutations in the genes encoding the α- or β-subunit of ETF or ETFDH, other as yet unidentified exogenic factors may play an important role in the initiation of this disease. Since

a proper mitochondrial flavin balance is maintained by a mitochondrial FAD transporter, a defect of this transporter or its precursor riboflavin could also cause a MADD-like phenotype [33].

In conclusion, a new type of equine acute myopathy is described. This may stimulate the performance of more metabolic investigations on equines suffering from rhabdomyolysis.

There are several reports concerning treatment of MADD in humans [17,34–36]. Biochemical MADD in horses might be of importance with reference to treatment options in the elusive types of human riboflavin-responsive MADD as well as to study the function of the mitochondrial FAD transporter in (equine) patients.

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