SHORT REPORT

ABSTRACT: Abnormal accumulations of lipid droplets, localized predominantly in histochemical type 1 fibers, were observed in fresh frozen sections of muscle biopsies from 25 dogs with myalgia, weakness, and muscle atrophy. Compared to controls, lactic acidemia, hyperalaninemia, lactic and pyruvic aciduria, variably increased urinary excretion of carnitine esters, and muscle carnitine deficiency were present. These findings support a metabolic block in oxidative metabolism resulting in lactic acidemia in dogs with lipid storage myopathy.
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ANALYSIS OF ORGANIC ACIDS, AMINO ACIDS, AND CARNITINE IN DOGS WITH LIPID STORAGE MYOPATHY

G. DIANE SHELTON, DVM, PhD,[1] WILLIAM L. NYHAN, MD, PhD,[2] PHILIP H. KASS, DVM, PhD,[4] BRUCE A. BARSHOP, MD, PhD,[2] and RICHARD H. HAAS, MA, MB, BChir[2,3]

[1] Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, California 92093-0612, USA
[2] Department of Pediatrics, School of Medicine, University of California, San Diego, La Jolla, California 92093-0612, USA
[3] Department of Neurosciences, School of Medicine, University of California, San Diego, La Jolla, California 92093-0612, USA
[4] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, California, USA

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A lipid storage myopathy is one in which abnormal amounts of lipid accumulate in muscle in a pattern which correlates with the oxidative capacity of muscle fibers. The accumulation of lipid represents the predominant pathological alteration. Most muscle pathologists evaluate the lipid content of a muscle biopsy specimen subjectively, using visual inspection of specimens stained for neutral triglycerides with oil red O or Sudan black.[5] Normal human muscle fibers contain sparse lipid deposits.[5] Observations of increased intramyofiber lipid has provided the initial diagnostic clue prompting further biochemical studies that led to the discovery of a metabolic defect.[5,7] Several disorders of lipid metabolism have characteristic patterns of urinary organic acid excretion that are detected using gas chromatography–mass spectrometry.[10,11] Further, excessive accumulation of acyl-CoA esters in the mitochondria may result in deficiency of carnitine as a result of formation of acyl-carnitine esters which exit from the cell and are preferentially excreted in the urine.[8] In this study, intermediates of major metabolic pathways were evaluated in 25 dogs with a histologic diagnosis of lipid storage myopathy by quantification of urinary organic acids, plasma amino acids, and plasma, urine, and muscle carnitine. Values from these dogs were compared with neuromuscular control and normal dogs.

Key words: canine; myopathy; lipid storage; lactic acidemia; carnitine

Correspondence to: Dr. G. Diane Shelton

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PATIENTS AND METHODS

Diagnostic muscle biopsy specimens, urine, and plasma were evaluated from 25 dogs of various breeds and ages and of both sexes having clinical signs of myalgia, weakness, and muscle atrophy. The vastus lateralis muscle was biopsied, placed in a water-tight container, and shipped at 4°C. Specimens were received within 24–36 h of sample collection. Dogs were screened for obesity, ischemic or renal disease, and previous treatment with valproic acid.[5] Muscle biopsies were flash frozen in isopentane precooled in liquid nitrogen and processed by a standard panel of histologic and histochemical stains and reactions.[6] Plasma samples were collected in sodium fluoride/potassium oxalate tubes and assayed

for lactate concentration using an immobilized enzyme membrane system (YSI Inc., Yellow Springs, OH). Urinary organic acids were quantified by gas chromatography–mass spectrometry (GC/MS) as described by Hoffman et al.[9] Plasma amino acids were quantified by automated column chromatography by the method of Spackman et al.[13] Urine, plasma, and muscle concentrations of total, free, and esterified carnitine were determined by radioisotopic enzyme assay by the method of Bieber and Lewin.[2] For comparison, muscle, urine, and plasma were collected from 10 adult mixed-breed dogs divided by gender without clinical evidence of neuromuscular disease, and 10 dogs with clinical neuromuscular disease and pathologic diagnoses of denervation, inflammatory myopathy, or various noninflammatory myopathies. Handling of specimens replicated the lipid storage myopathy group. A Kruskal–Wallis analysis of variance was used to compare data regarding organic and amino acid analysis between the three groups, along with a post hoc test of individual group differences adjusted for multiple comparisons.[3] A Mann–Whitney test for unpaired data was used to compare the carnitine concentrations between the lipid storage myopathy and neuromuscular control groups.[4]

RESULTS

Resting lactic acidemia (range 3–13.9 mmol/L; reference 0.5–2.0 mmol/L) was present in all dogs with lipid storage myopathy. Numerous lipid droplets (Fig. 1A), primarily within type 1 fibers and some type 2A fibers (Fig. 1B), were present within muscle biopsy specimens, and the lipid accumulation was the predominant pathological finding. Neither ragged red fibers nor other pathological abnormalities were observed. In comparison, absent or barely detectable lipid staining within type 1 and type 2A fibers (Fig. 1C, D) was observed within muscle biopsies from 10 dogs without clinical evidence of neuromuscular disease, and all plasma lactate levels were below 2.0 mmol/L. In the neuromuscular control group, lipid droplets were variably increased in 4/10 dogs, and mild lactic acidemia was present in 1/10 dogs. Unlike the biopsies with lipid storage myopathy, other pathological abnormalities predominated, and the amount of lipid observed was subjectively less.

Urinary L-lactic (mean 1156; median 1044; SD 562; reference <211 mmol/mol creatinine) and pyruvic (mean 184; median 179; SD 83; reference <39 mmol/mol creatinine) acid excretion were significantly elevated ($P < 0.05$), as was excretion of the short chain fatty acids 2-OH butyric, 3-OH butyric, and 3-OH isobutyric. The dicarboxylic acids adipic and sebacic were not present in the urine of any of the dogs tested. Although there was a significant difference in level of suberic acid ($P < 0.05$) compared to the control groups, this was not marked. Plasma alanine was elevated in 21/25 dogs (mean 1101; median 999; SD 612; reference 376–672 μmol/L) and was significantly different from both control groups ($P < 0.05$).

Total and free plasma carnitine was low in only 2/25 dogs. Urinary excretion of total carnitine and carnitine esters was increased in 23/25 dogs. Elevated urinary excretion of total carnitine (6/10) and free carnitine (7/10) was also found in neuromuscular controls. In the lipid myopathy group, urinary excretion of total carnitine and carnitine esters was, however, significantly higher than the neuromuscular control group ($\alpha = 0.05$). Total muscle carnitine levels were low in 13/25 dogs with lipid storage myopathy and in 4/10 dogs with other neuromuscular diseases. Free muscle carnitine was low in 14/25 and 5/10 dogs with lipid storage myopathy and other neuromuscular disorders, respectively.

DISCUSSION

Lactic acidemia, lactic and pyruvic aciduria, and hyperalaninemia are consistent with a block in oxidative metabolism. When a defect is present in either the pyruvate dehydrogenase complex or electron-transport chain, pyruvate is metabolized to either lactate by lactate dehydrogenase or to alanine by alanine aminotransferase. In most cases of electron-transport chain defects, the redox state is altered due to reduced nicotinamide adenine dinucleotide accumulation, resulting in lactic acidemia. Impaired muscle oxidative metabolism has also been suggested in human patients with exercise intolerance of undetermined cause based on phosphorus magnetic resonance spectroscopy.[1] β-Oxidation defects and primary disorders of carnitine metabolism are unlikely causes of the lactic acidemia and lipid storage myopathy in these dogs. Impairment of the pyruvate dehydrogenase (PDH) complex is possible in some dogs, since alanine is markedly elevated in disorders of the PDH complex, and plasma alanine levels were markedly elevated (>1500 μmol/L, reference 450–672) in 4/25 dogs. A respiratory chain defect is also possible in some dogs. Muscle carnitine deficiency was probably secondary to formation of acyl-carnitine esters excreted in the urine.

The results of this study support the presence of one or more disorders of mitochondrial oxidative metabolism in dogs associated with lipid storage my-

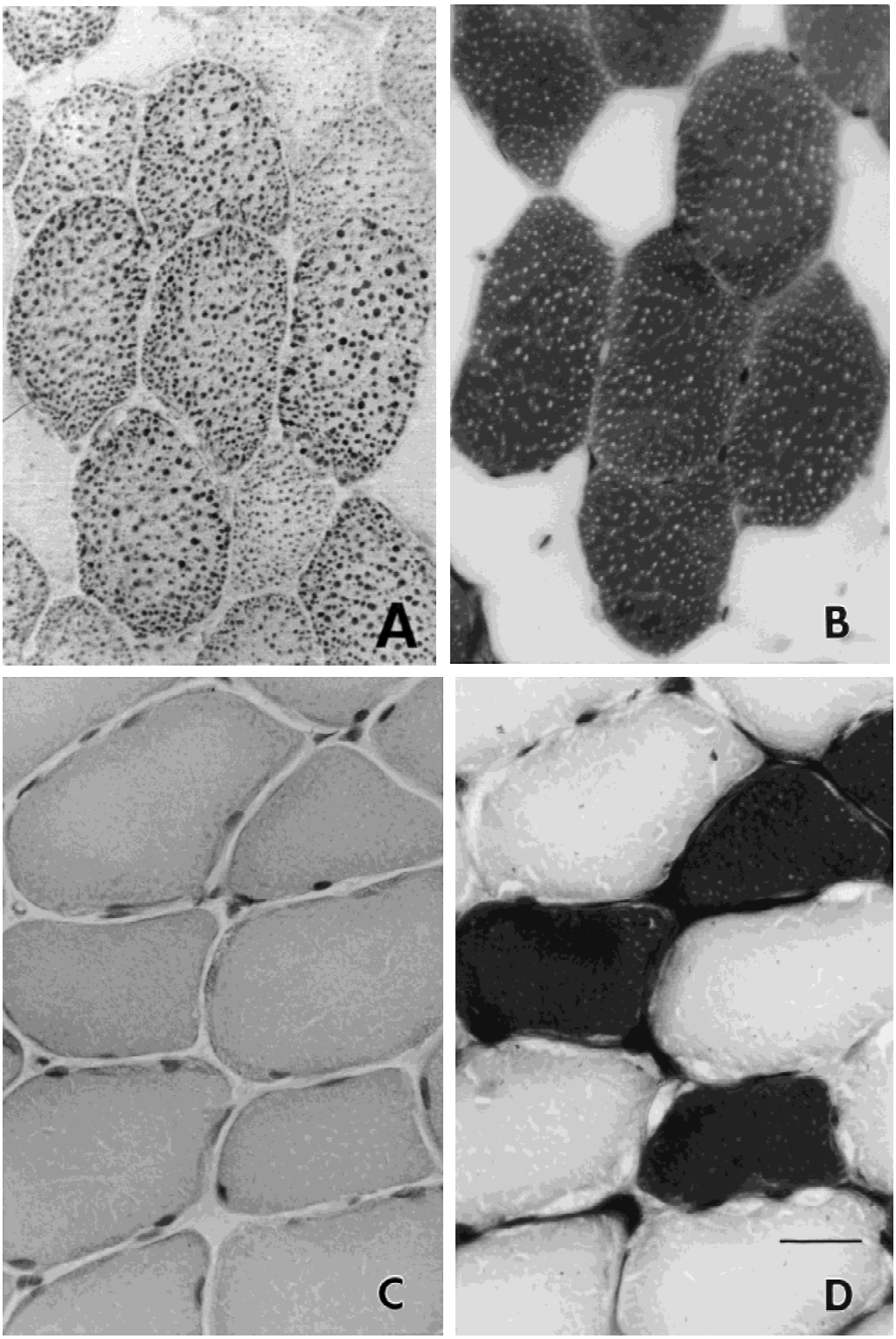

FIGURE 1. Fresh frozen biopsy sections from the vastus lateralis muscle of a dog with lipid storage myopathy **(A, B)** and from a reference dog without clinical signs of neuromuscular weakness or lactic acidemia **(C, D).** Paired serial sections from the dog with lipid storage myopathy illustrates the presence of numerous lipid droplets using the oil red O stain **(A)** and localization of lipid to predominantly type 1 (dark staining) and some type 2A (light staining) fibers using the myofibrillar adenosine triphosphatase reaction preincubated at pH 4.3 **(B).** By contrast, the muscle biopsy from the reference dog had no demonstrable lipid droplets **(C, D).** Bar = 38 μm.

opathy. Mild lactic and pyruvic aciduria in the absence of intramyofiber lipid droplets was present in 1 neuromuscular control dog with hypothyroidism and steroid myopathy. Since ultrastructural abnormalities, including mitochondrial aggregation, swelling, and vacuolization, have been described in experimental corticosteroid myopathy,[12] the presence of mitochondrial-related metabolic abnormalities may be expected in clinical steroid myopathy.

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