Pyruvate dehydrogenase deficiency in a Sussex spaniel

A two-year-old, intact female Sussex spaniel was presented with signs of exercise intolerance. Pre- and post-exercise serum lactate and pyruvate concentrations and urinary organic acid screening supported a diagnosis of pyruvate dehydrogenase deficiency, as previously reported in this breed. Dietary therapy was initiated for six months, during which time there was no reported clinical deterioration. A full neurological examination and repeat evaluation of lactate and pyruvate concentrations before and after exercise was conducted one year after diagnosis, at which time the patient had been without dietary modification for six months and had developed more severe exercise intolerance along with evidence of central nervous system dysfunction.

C. J. Abramson, S. R. Platt
and G. D. Shelton*

Centre for Small Animal Studies,
Animal Health Trust, Suffolk
CB8 7UU

*Department of Pathology, University
of California, San Diego, La Jolla,
CA 92093-0709, USA

C. J. Abramson's current address is
the Department of Veterinary
Clinical Sciences, The Ohio State
University, GOI Vernon L. Tharp
Street, Columbus, OH 43210, USA

INTRODUCTION

Pyruvate dehydrogenase (PDH) complex deficiency stems from a group of inherited disorders which result in defective activity of the PDH enzyme complex. This enzyme complex is a necessary part of glucose metabolism which helps to metabolise pyruvate into acetylcoenzyme A (acetyl-CoA) in the cell mitochondria. Acetyl-CoA is utilised to produce energy in the form of adenosine 5'-triphosphate (ATP) via the tricarboxylic acid cycle. An inability to metabolise pyruvate into acetyl-CoA results in the pyruvate being metabolised into lactate, which may lead to lactic acidosis. In humans, it is known to be an X-linked inherited disease and one of the most common genetic defects affecting mitochondrial substrate utilisation. It has a very heterogeneous clinical presentation and severity in humans which varies from fatal lactic acidosis in newborns to chronic seizures and intermittent ataxia (Brown and others 1994, Cross and others 1994, Wexler and others 1997).

Pyruvate dehydrogenase deficiency has previously been reported in Sussex and Clumber spaniels in the UK and USA through published correspondence, short communications and abstracts (Herrtage and Houlton 1979, Houlton and Herrtage 1980, Shelton and others 2000). However, this report is the first complete case description and long-term evaluation of a Sussex spaniel with PDH deficiency. Details of recommended dietary therapy are discussed.

CASE HISTORY

A 22-month-old, intact female Sussex spaniel was referred to the neurology service at the Animal Health Trust for investigation of exercise intolerance and post-exercise collapse. The dog had begun displaying exercise intolerance at three months of age, which commenced as an inability to walk further than approximately 10 m before collapsing on all four limbs. There was some improvement as the dog matured, and by one year of age it was able to walk up to 350 m before progressing to episodic collapse. The time between collapsing episodes decreased as physical activity continued, and the time needed for recovery increased. Calcium supplementation and carprofen (Rimadyl; Pfizer), 2 mg/kg bodyweight twice daily for five days, had been administered with no clinical improvement noted.

The dog was initially referred to a veterinary cardiologist for suspected syncopal episodes. The cardiologist diagnosed aortic valve incompetence as a consequence of aortic valve dysplasia; however, a complete cardiac evaluation suggested that the collapse was unlikely to be due solely to the dog's heart defect. The dog was then referred for neurological evaluation.

At the time of referral, the only abnormality noted on general physical examination was mild left-sided hip joint laxity. The findings on neurological examination were within normal parameters. The owners provided video footage of the dog at exercise that showed intermittent collapse on all four limbs which became progressively more frequent as the exercise period progressed. The problem was localised to the peripheral neuromuscular system.

Table 1. Lactate and pyruvate levels before and after exercise

	Normal*	Initial testing	One year evaluation
Pre-exercise lactate	0·7-2·0 mmol/litre	6·02 mmol/litre	7·62 mmol/litre
Pre-exercise pyruvate	0·06-0·10 mmol/litre	1·25 mmol/litre	1·06 mmol/litre
Pre-exercise L:P	8·8-25·2	4·8	7·2
Post-exercise lactate	1·35-5·8 mmol/litre	16·26 mmol/litre	9·97 mmol/litre
Post-exercise pyruvate	0·11-0·27 mmol/litre	2·89 mmol/litre	2·88 mmol/litre
Post-exercise L:P	14·6-26·4	5·6	3·5
Post-rest lactate†	1·8-4·8 mmol/litre	7·93 mmol/litre	4·44 mmol/litre
Post-rest pyruvate†	0·13-0·221 mmol/litre	1·23 mmol/litre	0·557 mmol/litre
Post-rest L:P†	15·5-27	6·4	7·9

*References for normal range from Matwichuk and others (1999)
†Post-rest values taken after 10 minutes rest
L:P Lactate:pyruvate ratio

Chest radiographs at the time of cardiac referral revealed a mild cardiomegaly which had been non-progressive over an eight-month period of time. Hip radiographs from the referring veterinary practice revealed no evidence of degenerative joint disease. Routine haematology and serum biochemistry were unremarkable. The serum acetylcholine receptor antibody titre to test for myasthenia gravis was within normal parameters at 0·06 nmol/litre (reference <0·6 nmol/litre).

General anaesthesia was induced with propofol (Rapinovet; Schering-Plough Animal Health) given intravenously to effect (to a maximum dose of 4 mg/kg bodyweight) and maintained with halothane gas (Fluothane; Schering-Plough Animal Health) to permit electrodiagnostic testing, muscle biopsy and nerve biopsy. Electromyography of limb, paraspinal and head muscles showed no abnormal spontaneous electrical discharge activity. Motor nerve conduction studies on the sciatic-tibial nerve demonstrated average conduction times of 73 m/second (reference range 69·4 ± 1·3 m/second) (Redding and others 1982). Repetitive stimulation of the sciatic-tibial nerve was considered within normal parameters.

Fresh and glutaraldehyde-fixed biopsies from the biceps femoris and gastrocnemius muscles were evaluated using a standard panel of histological and histochemical stains and enzyme reactions (Dubowitz 1985). No abnormalities were identified. Muscle carnitine concentration was markedly decreased (total carnitine 1 mmol/mg protein, reference range 12 to 41 mmol/mg; free carnitine 1 mmol/mg protein, reference range 11 to 33 mmol/mg). Urine carnitine concentrations were increased (total carnitine 54·7 mmol/mol creatinine; reference range 0 to 32 mmol/mol carnitine; free carnitine 30·5 mmol/mol creatinine; reference range 0 to 15 mmol/mol carnitine) (Bierber and Lewin 1981, Shelton and others 1998). Fresh and glutaraldehyde-fixed biopsies of the peroneal nerve were also examined and found to be histologically normal. Skin biopsies were taken for fibroblast culture and subsequent measurement of pyruvate dehydrogenase enzyme activity, but the cultures were unsuccessful due to fungal contamination in the laboratory.

Exercise evaluation was conducted with serum lactate and pyruvate levels measured before, immediately after 10 minutes of running, and 10 minutes after cessation of running. All samples were analysed as described by Matwichuk and others (1999). Markedly elevated lactate and pyruvate concentrations were documented at all time points, with a lactate:pyruvate ratio of less than 10 (Table 1). These findings are consistent with PDH deficiency, as previously reported in the Sussex spaniel breed (Houlton and Herrtage 1980, Shelton and others 2000). Urinary organic acid analysis by gas chromatography-mass spectroscopy was performed at the Biochemical Genetics Laboratory, University of California, San Diego, using techniques described by Hoffman and others (1989). There was markedly elevated urinary excretion of pyruvic acid (588 mmol/mol creatinine, reference range 0 to 26 mmol/mol creatinine) (Shelton and others 1998), which further supported the preliminary diagnosis of PDH deficiency.

Supplementation with L-carnitine (Carniking Powder; Lohmen Animal Health), 1 g twice daily with food, was initiated due to the documented low concentrations of muscle carnitine. The diet was changed to a high-fat, low-carbohydrate food source (Hill's Science Diet n/d) with the addition of thiamine (Holland & Barret), 100 mg/day orally, as is advocated in human cases of PDH deficiency (Brown and others 1994, Wexler and others 1997). This diet was maintained for six months after which financial concerns led the owners to discontinue the dietary regimen. The owners reported that there had been no significant change in the patient's status while the dog was on the diet.

Six months after discontinuing the diet, the patient was re-presented to the neurology service for evaluation. At this time, the owners reported that the dog was having more severe episodes of collapse which required up to 45 minutes of rest for complete recovery. Neurological examination revealed a normal posture and gait, although physical activity was only maintained for two minutes before the dog became recumbent and required a five-minute period of rest before rising again. Deficits in proprioception were noted in the right pelvic limb. Cranial nerve examination revealed bilaterally poor menace responses and a positional ventral strabismus in the right eye. The neurological examination was consistent with a multifocal brain lesion involving the vestibular, proprioceptive and menace pathways, possibly arising from progression of the metabolic disease. Other differential diagnoses included sterile inflammatory disease, infectious disease or neoplasia. Magnetic resonance imaging of the brain and brainstem was recommended, but the owners declined.

Repeat evaluation of lactate and pyruvate levels before exercise, immediately after exercise, and after a 10 minute rest from exercise, revealed similar elevations to those seen at initial presentation (Table 1). Venous blood gas testing after exercise revealed a metabolic acidosis with respiratory compensation (pH 7·38, HCO₃ 14·6, base excess –10·5, pCO₂ 25·4). The owners declined a recommendation to place the dog back onto the low-carbohydrate, high-fat diet with supplementation of carnitine and thiamine, as previously prescribed.

DISCUSSION

The diagnosis of PDH deficiency in this case was made based on lactic acidaemia with a lactate:pyruvate ratio of less than 10 (Shelton and others 2000). Although confirmation of the disorder is ideally based on PDH activity measurements in cultured fibroblasts, this was not possible in this patient due to fungal contamination in the laboratory. In humans, PDH deficiency is diagnosed using enzyme assay in cell/tissue culture or cDNA/genomic sequencing (Lib and others 2002). DNA diagnosis of this disorder has not yet been established in dogs as the specific genetic defect has, to date, not been characterised in dogs.

In human cases, PDH deficiency affects all tissues of the body, having a particular clinical effect on the brain due to the brain's dependency on aerobic oxidation of glucose for energy. There is a large phenotypic variability in the disorder in humans due to the variety of genetic anomalies that lead to abnormalities in the PDH complex and the phenomenon of X chromosome inactivation in females (Brown and others 1989, Lib and others 2002). A severe neonatal lactic acidaemia more commonly affects males, while more chronic neurological forms resulting in mental retardation, microcephaly, blindness and spasticity more commonly affect females (Brown and others 1989, Cross

and others 1994, Lissens and others 2000). Structural brain changes, including cerebral atrophy, ventricular dilation, and malformation of the corpus collosum, medullary pyramids and inferior olives, have also been documented in humans with this disease (Brown and others 1994).

The predominant clinical sign in previously reported cases of PDH deficiency in Sussex spaniels was exercise intolerance. Without published follow-up, it is not possible to comment on the progression of signs or chronicity of the lesions in these dogs, although no central nervous system signs have previously been reported. The patient in this case report also presented with exercise intolerance as the primary clinical sign. The clinical signs were reported to be stable for the first six months post-diagnosis while the dog was maintained on dietary therapy. However, there was progressive deterioration in exercise tolerance thereafter and the development of central nervous system signs. Unfortunately, the owners declined further diagnostic work-up for the central nervous system signs, and it is therefore impossible to confirm at this time that the signs were directly related to progression of the PDH deficiency.

There is no standard treatment for PDH deficiency in humans. Several sources recommend a ketogenic diet. This diet of high fat and low carbohydrate food sources leads to the development of ketones in the body which can be metabolised by the brain for energy without utilising the pathway involving PDH. Decreasing the carbohydrate metabolism also decreases the production of lactate and may be protective against lactic acidosis. The heterogeneous phenotype of PDH deficiency and the heterogeneity of the fat, carbohydrate and protein content among 'ketogenic diets' in the literature makes it difficult to fully assess the efficacy of this therapy in human patients (Blass and Gibson 1978, Brown and others 1994, Wexler and others 1997).

The authors are not aware of any canine patients that have been documented to maintain ketosis on high-fat, low-carbohydrate diets, and the use of diets in an attempt to induce ketosis may not be successful in this species. However, a high-fat (52 per cent) and low-carbohydrate diet has previously been used with carnitine and thiamine supplementation to produce clinical improvement in a Clumber spaniel with PDH deficiency (Shelton and others 2000). Thus, dietary change to a high-fat, low-carbohydrate diet was recommended for the patient in this report. After a review of available diets, the dog was placed onto Hill's Science Diet n/d (moist, 37·2 per cent protein, 32·1 per cent fat, and 21·5 per cent carbohydrate) (Hand and others 2000).

Supplementation with oral carnitine, a 'conditionally essential' nutrient, is warranted for any animal placed onto a high-fat diet. Carnitine is an amino acid that is required for beta-oxidation of long-chain fatty acids. It helps to transport long-chain fatty acids into mitochondria and is an essential component of fat energy production (Carroll and Cote 2001). Sustained fatty acid oxidation can result in increased excretion of urinary acylcarnitine and leads to carnitine deficiency (Hommes and others 1973). Thus, monitoring of carnitine levels in humans with PDH deficiency is recommended, and dietary supplementation is provided as needed. Supplementation was particularly relevant in this case due to the documented low concentrations of muscle carnitine. Thiamine supplementation is also recommended for PDH deficiency in humans, as clinical improvement has been documented with supplementation (Hommes and others 1973, Brown and others 1994). This effect does not seem to be due to a direct thiamine or thiamine pyrophosphate dependency of the PDH complex defect, but is more likely to result from the elevated levels of thiamine pyrophosphate causing activation of residual PDH complex activity (Hommes and others 1973).

Conclusions

Although the owners reported no clinical deterioration while this patient was maintained on a high-fat, low-carbohydrate diet with thiamine and carnitine supplementation, there was no reassessment of the lactate:pyruvate ratios to confirm a positive effect of the dietary changes on the metabolic abnormalities in PDH deficiency. Further evaluation of the high-fat, low-carbohydrate diet currently recommended for the treatment of PDH deficiency is warranted. The monitoring of clinical and neurological status as well as lactate and pyruvate levels of patients maintained on this diet is needed in the future to validate it as an effective treatment for PDH deficiency in dogs.

Acknowledgements

References

BIERBER, L. L. & LEWIN, L. M. (1981) Measurement of carnitine and O-acylcarnitines. *Methods in Enzymology* **72**, 276-281

BLASS, J. P. & GIBSON, G. E. (1978) Studies of the pathophysiology of pyruvate dehydrogenase deficiency. *Advances in Neurology* **21**, 181-194

BROWN, G. K., BROWN, R. M., SCHOLEM, R. D., KIRBY, D. M. & DAHL, H. H. M. (1989) The clinical and biochemical spectrum of human pyruvate dehydrogenase complex deficiency. *Annals of the New York Academy of Science* **573**, 360-368

BROWN, G. K., OTERO, L. J., LEGRIS, M. & BROWN, R. M. (1994) Pyruvate dehydrogenase deficiency. *Journal of Medical Genetics* **31**, 875-879

CARROLL, M. C. & COTE, E. (2001) Carnitine: A review. *Compendium on Continuing Education for the Practicing Veterinarian* **23**, 45-51

CROSS, J. H., CONNELLY, A., GADIAN, D. G., KENDALL, B. E., BROWN, G. K., BROWN, R. M. & LEONARD, J. V. (1994) Clinical diversity of pyruvate dehydrogenase deficiency. *Pediatric Neurology* **10**, 276-283

DUBOWITZ, V. (1985) Muscle Biopsy: a Practical Approach. Baillière Tindall, London. pp xx-xx

HAND, M. S., THATCHER, C. G., REMILLARD, R. L. & ROUDEBUSH, P. (2000) Small Animal Clinical Nutrition, 4th edn. Mark Morris Institute, Topeka. pp 88-89, 898

HERRTAGE, M. E. & HOULTON, J. E. F. (1979) Collapsing Clumber spaniels (Letter). *Veterinary Record* **105**, 334

HOFFMANN, G., ARAMAKI, S., BLUM-HOFFMANN, E., NYHAN, W. L. & SWEETMAN, L. (1989) Quantitative analysis for organic acids in biological samples: Batch isolation followed by gas chromatography-mass spectrometric analysis. *Clinical Chemistry* **35**, 587-595

HOMMES, F. A., BERGER, R. & LUIT-DE-HAAN, G. (1973) The effect of thiamine treatment on the activity of pyruvate dehydrogenase: relation to the treatment of Leigh's encephalomyelopathy. *Pediatric Research* **7**, 616-619

HOULTON, J. E. F. & HERRTAGE, M. E. (1980) Mitochondrial myopathy in the Sussex spaniel. *Veterinary Record* **106**, 206

LIB, M. Y., BROWN, R. M., BROWN, G. K., MARUSICH, M. F. & CAPALDI, R. A. (2002) Detection of pyruvate dehydrogenase E1α – subunit deficiencies in females by immunohistochemical demonstration of mosaicism in cultured fibroblasts. *Journal of Histochemistry & Cytochemistry* **50,** 877-884

LISSENS, W., DEMEIRLEIR, L., SENECA, S., LIEBAERS I., BROWN, G. K., BROWN, R. M., ITO, M., NAITO, E., KURODA Y., KERR, D. S., WEXLER, I. D., PATEL, M. S., ROVINDON, H. & SEYDA A. (2000) Mutations in the X-linked pyruvate dehydrogenase (E1) α subunit gene (PDHA1) in patients with a pyruvate dehydrogenase complex deficiency. *Human Mutation* **15**, 209-219

MATWICHUK, C. L., TAYLOR, S. M., SHMON, C. L., KASS, P. H. & SHELTON, G. D. (1999) Changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values in healthy Labrador Retrievers before and after strenuous exercise. *American Journal of Veterinary Research* **60**, 88-92

REDDING, R. W., INGRAM, J. T. & COLTER, S. B. (1982) Sensory nerve conduction velocity of cutaneous afferents of the radial, ulnar, peroneal, and tibial nerves of the dog. *American Journal of Veterinary Research* **43**, 517-521

SHELTON, G. D., NYHAN, W. L., KASS, P. H., BARSHOP, B. A. & HASS, R. H. (1998) Analysis of organic acids, amino acids, and carnitine in dogs with lipid storage myopathy. *Muscle & Nerve* **21**, 1202-1205

SHELTON, G. D., VAN HAM, L., BHATTI, S., COOK-OLSON, S., JOHNSON, K., BARSHOP, B., TOONE, J. & APPLEGARTH, D. (2000) Pyruvate dehydrogenase deficiency in Clumber and Sussex Spaniels in the United States and Belgium. *Journal of Veterinary Internal Medicine* **14**, 342

WEXLER, I. L., HEMALATHA, S. G., MCCONNELL, J., BUIST, N. R. M., DAHL, H. H. M., BERRY, S. A., CEDERBAUM, S. D., PATEL, M. S. & KERR, D. S. (1997) Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. *Neurology* **49**, 1655-1661