Acknowledgements

The authors would like to thank Linda Horspool and Kirsty Reid for their assistance in this study.

References

Bogan, J. A., Mohammed-Ali, N. A. K. and Formica, G. (1985) Pharmacokinetic studies with the triclabendazole/fenbendazole combination in sheep and cattle. *World Association for the Advancement of Veterinary Parasitology Abstracts, Rio de Janeiro, Brazil.*, **165**, 38.

Boray, J. C., Strong, M. B. and Allison, J. R. (1981) Chemoprophylaxis of fascioliasis. *Australian Society of Parasitology*, **Abst 20.**

Boray, J. C., Crowfoot, P. D., Strong, M. B., Allison, R. R., Schellenbaum, M., Von Orelli, M. and Sarasin, G. (1983) Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Vet. Rec.*, **113**, 315-317.

Gibaldi, M. and Perrier, D. (1975) *Pharmacokinetics. Dekker, New York*, pp 329.

Hennessy, D. R., Lacey, E., Steel, J. W. and Prichard, R. K. (1987) The kinetics of triclabendazole disposition in sheep. *J. vet. Pharmacol. Therap.*, **10**, 64-72.

Kinabo, L. D. B. and Bogan, J. A. (1988) Pharmacokinetics and efficacy of triclabendazole in goats with induced fascioliasis. *J. vet. Pharmacol. Therap.*, **11**, 254-259

Lancaster, R. (1980) Pharmacology in Clinical Practice. William Heinmann Medical Books Ltd, London, pp 31-48.

Marriner, S. E. and Bogan, J. A. (1980) Pharmacokinetics of albendazole in sheep. *Am. J. vet. Res.*, **41**, 1126-1129.

Marriner, S. E. and Bogan, J. A. (1981) Pharmacokinetics of fenbendazole in sheep. *Am. J. vet. Res.*, **42**, 1146-1148.

Marriner, S. E. and Bogan, J. A. (1985) Plasma concentrations of fenbendazole and oxfendazole in the horse. *Equine vet. J.*, **17**, 58-61.

Mohammed-Ali, N. A. K., Bogan, J. A., Marriner, S. E. and Richards, R. J. (1986) Pharmacokinetics of triclabendazole alone or in combination with fenbendazole in sheep. *J. vet. Pharmacol. Therap.*, **9**, 442-445.

Sedman, A. J. and Wagner, J. G. (1976) CSTRIP, a Fortran IV computer program for obtaining initial polyexponential parameter estimates. *J. Pharm. Sci.*, **65**, 1006-1010.

Turner, K., Armour, J. and Richards, R. J. (1984) Anthelmintic efficacy of triclabendazole against *Faciola hepatica* in sheep. *Vet. Rec.*, **114**, 41-42.

Wolff, K., Eckhert, J., Schneiter, G. and Lutz, H. (1983). Efficacy of triclabendazole against *Fasciola hepatica* in sheep and goats. *Vet. Parasitol.*, **13**, 145-150.

Received for publication 11.3.88
Accepted 22.6.88

EQUINE VETERINARY JOURNAL
Equine vet. J. (1989) **21** (4) 307-308

Normal resting values of plasma free carnitine and acylcarnitine in horses predisposed to exertional rhabdomyolysis

R. VAN DEN HOVEN* and H. J. BREUKINK
Clinic of Large Animal Medicine, University of Utrecht, Yalelaan 16, 3584 CH Utrecht, The Netherlands.

M. H. V. VAANDRAGER-VERDUIN and H. R. SCHOLTE
Department of Biochemistry II, Erasmus University, Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

A. E. F. H. MEIJER
Department of Experimental Neurology, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Introduction

CARNITINE is indispensable for the oxidation of long-chain fatty acids by the mitochondria. These fatty acids are activated on the outer face of the mitochondrial outer membrane or the reticulum (Groot, Scholte and Hülsmann 1976). The acyl-CoA produced is converted into acylcarnitine by carnitine palmitoyltransferase I (E.C.2.3.1.21) localised on the inner face of the outer mitochondrial membrane (Murthy and Pande 1987). Acylcarnitine is transported over the mitochondrial membrane by the carnitine carrier, and transformed into acyl-CoA by carnitine palmitoyltransferase II on the inner face of the inner mitochondrial membrane (Bremer 1983). Acyl-CoA is further oxidised by the mitochondrial beta-oxidation system and the Krebs cycle to $C0_2$, H_20 and energy.

It is likely that in herbivorous animals such as the horse, consuming diets low in carnitine, carnitine is synthesised by the animal itself. This occurs by a multi-step biosynthetic pathway from lysine and S-adenosyl-methionine via deoxycarnitine to carnitine. The enzyme catalysing the last step is localised in the liver of most animals, and also in the kidney of primates (Scholte and de Jonge 1987).

In only a few human subjects with exercise-induced myoglobinuria, enzymatic defects were found in carbohydrate or lipid catabolism (Rowland 1984). In human pathology, carnitine deficiency is frequently encountered (Borum 1986; a Gitzelmann, Baerlocher and Steinmann 1987), but myoglobinuria is only seldom described in these patients, probably because muscular weakness, hepatic encephalopathy or cardiomyopathy dominate

R. VAN DEN HOVEN'S present address is Duphar BV, Animal Health Department, C. J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands.

Table I: Plasma free and acylcarnitine concentrations in (μmol/l) in horses predisposed to exertional rhabdomyolysis (ER) and healthy horses

Horses predisposed to ER			Healthy horses		
	Carnitine			Carnitine	
	free	acyl		free	acyl
5-year-old mare	21.2	7.3	3-year-old mare	21.0	4.7
6- year-old mare	21.1	3.8	4-year-old mare	23.8	2.1
10-year-old gelding	20.0	5.6	5-year-old mare	22.1	6.5
3-year-old mare	19.6	5.8	3-year-old gelding	23.6	6.1
7-year-old gelding	27.2	4.3	8-year-old gelding	28.4	6.6
3-year-old mare	19.1	5.3			
	$\bar{x}$=21.4	$\bar{x}$=5.2		$\bar{x}$=23.8	$\bar{x}$=5.2
	sd=3.0	sd=1.2		sd=2.8	sd=1.9

the disease. In exercising horses a defect in carbohydrate catabolism is unlikely because their outstanding athletic ability depends on glycolytic energy. We wondered, therefore, if the gradual change-over to oxidative phosphorylation is hampered in the myoglobinuric or tied-up horse by a persistent lesion in the carnitine system or another mitochondrial defect. Because prevailing mitochondrial defects in man, such as acyl-CoA dehydrogenase deficiencies and respiratory chain lesions, are often accompanied by a decrease in free carnitine and an increase in acylcarnitine in plasma and urine, we studied both free and total carnitine levels in plasma of physically inactive horses with previous episodes of exertional rhabdomyolysis.

Materials and methods

Plasma free carnitine and acylcarnitine concentrations were determined in six horses with histories of repeated attacks of exertional rhabdomyolysis but which were free of clinical signs at the time of sampling; and in five clinically healthy horses (Table 1). Plasma CK, AST and LDH activity were determined according to standard methods. Blood was collected in heparinised tubes from the jugular vein. Immediately after collection the samples were centrifuged for 10 mins at 2000 g. The plasma was collected and frozen at -20°C until analysis. Carnitine was analysed as described by Barth *et al.* (1983) Wilcoxon rank test was used for statistical analysis of the figures.

Results and Discussion

Plasma CK, AST and LDH activity of all horses were within normal ranges. Plasma concentration of free carnitine and acylcarnitine for the two groups of horses are shown in Table 1. In healthy horses the range of free carnitine was 21.0 to 28.4 μmol/litre and for acylcarnitine 2.1 to 6.6 μmol/litre. In horses predisposed to exertional rhabdomyolysis the free carnitine range was 19.1 to 27.2 μmol/litre and the acylcarnitine ranges was 3.8 to 7.3 μmol/litre. The Wilcoxon rank test did not show any significant difference in free carnitine ($P>0.10$) and acylcarnitine ($P>0.50$) between horses predisposed to exertional rhabdomyolysis and normal horses. The free carnitine concentrations in plasma in this study are in agreement with the findings of Foster and Harris (1986).

Compared to man in which the concentration range of free carnitine and total carnitine is 25 to 64 μmol/litre (n=71) and 29 to 74 μmol/litre (n=37) respectively, horses show a remarkably low concentration irrespective of whether they are clinically normal or predisposed to exertional rhabdomyolysis. The horse possesses a highly efficient muscular uptake of plasma carnitine to obtain adequate concentrations in the musculature. It has been demonstrated in healthy horses that the plasma concentration of free carnitine is approximately 1/300 of that in the muscles (Foster and Harris 1986). Available data suggest neither a systemic carnitine deficiency as described for man (Karpati *et al* 1975), nor a defective mitochondrial metabolism of acyl-CoA ester at rest as a likely cause for the disease.

In healthy horses Foster and Harris (1986) demonstrated that the concentration of plasma free carnitine was not affected immediately by exercise, but tended towards lower concentrations during a 70 min standing period. Whether this pattern is changed in horses predisposed to exertional myopathy has to be studied. Changes in plasma carnitine concentrations may indicate mitochondrial disturbances.

In order to find out if an intramuscular carnitine uptake defect may be associated with the predisposition to exertional rhabdomyolysis the carnitine system in muscles should be studied.

References

Barth, P. G., Scholte, H. R., Berden, J. S., van der Klei-van Moorsel, J. M., Luyt-Houwen, I. E. M., van 't Veer-Korthof, E. Th., van der Harten, J. J. and Sobotka-Plojhar, M. A. (1983). An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leukocytes. *J. neurol. Sci.*, **62**, 327-355.

Borum, P. G. (ed.) (1986). *Clinical Aspects of Human Carnitine Deficiency.* Pergamon Press, New York.

Bremer, J. (1983). Carnitine-metabolism and functions. *Physiol. Revs.* **63**, 1420-1479.

Foster, C. V. L., Harris, R. C. (1986). Changes in free and bound carnitine in muscle with maximal sprint exercise in the thoroughbred horse. *Equine Exercise Physiology* **2**, ICEEP Publ., Davis, California, pp. 332-340

Gitzelmann, R., Baerlocher, K. and Steinmann, B. (eds) (1987). Carnitine in der Medizin, Schattauer, Stuttgart.

Groot, P. H. E., Scholte, H. R. and Hülsmann, W. C. (1976). Fatty acid activation: specificity, localization and function. *Adv. Lipid Res.*, **14**, 75-126.

Karpati, G., Carpenter, S., Engel, A. G., Watters, G., Allen, J., Rothmann, S., Klassen, G., Mamer, O. A. (1975). The syndrome of systemic carnitine deficiency: Clinical, morphological, biochemical and pathophysiologic features. *Neurology (Mineap.)* **25**, pp. 16-24.

Murthy, M. S. R. and Pande, S. V. (1987). Malonyl-CoA binding site and the overt carnitine palmitoyltransferase activity reside on the opposite sides of the outer mitochondrial membrane. *Proc. Natl. Acad. Sci. U.S.*, **84**, 378-382.

Rowland, L. P. (1984). Myoglobinuria. Can. J. Neurol. Sci., 11, 1-13.

Scholte, H. R. and de Jonge, P. C. (1987). Mitochondrial myopathies In: *Carnitine in der Medizin.* Ed: R. Gitzelmann, K. Baerlocher and B. Steinmann. Schattauer, Stuttgart, pp 21-59.

Received for publication 26.8.87
Accepted 13.2.89