

Brief Communication

CK ISOZYMES IN SERUM OF HALOTHANE SENSITIVE
AND NON-REACTING PIGS *

Creatine phosphokinase — CK — is mainly formed in striated muscles, heart and nervous tissues. Being a dimer, CK exists in three forms, BB, MB and MM. BB isoenzyme is characteristic for nervous tissue, while MM is found in skeletal muscles. MB is present in heart tissue in addition to the MM isoenzyme; in porcine myocardium MM accounts for about 90 % of the CK activity.

Pigs developing porcine stress syndrome (PSS) are hypersensitive to halothane anesthesia. When subjected to this type of anesthesia piglets develop symptoms characterized by progressive hyperthermia, severe muscle rigidity and metabolic acidosis, which has been defined as malignant hyperthermia syndrome (MHS) (*McLoughlin 1977*). As most halothane sensitive swine are stress susceptible, halothane anesthesia has been used as a screening test for abnormal reactors.

Raised serum creatine phosphokinase (S-CK) levels have been demonstrated in pigs sensitive to halothane (*Eikelenboom & Minckema 1974*), and there are also reports of raised S-CK levels in humans reacting abnormally to halothane and suxamethonium.

Forty-nine pigs of Swedish landrace or crossbreeds of Swedish landrace × Yorkshire, 9—12 weeks old, females, males and castrates, were studied. All the piglets were tested with halothane anesthesia (5 % halothane in oxygen), inhaled by a mask for 5 min or until typical muscular reactions occurred. A progressive muscular rigidity is provoked within a few minutes in halothane sensitive pigs. Blood samples were drawn from anterior vena cava prior to the test procedure. The activity of CK in serum was measured according to CK activation reagent Kit (Boehringer, Mannheim). The CK isoenzymes were separated by agarose gel electrophoresis. The isoenzyme fractions were quantitated by use of fluorometric assay reagent (Corning, Kebo-Grave, Stockholm), and the isoenzyme activities were estimated by use of a densitometer for u.v. light scanning, and then calculated from the total CK activity in serum.

Twelve of the 49 pigs were classified as stress susceptible. The halothane sensitive pigs in this study had significant higher CK activity in serum than non-reacting pigs of corresponding

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Table 1. S-CK and CK isoenzymes, total activity and non-reacting

Piglets	CK total	MM	
		total	rel. %
Halothane reactants	26.7 ± 35 (6 — 124) 12	21.9 ± 27 (5 — 106) 12	88.3 ± 7.4 (68 — 95) 12
Non- reactants	5.6 ± 2.6 (2.4 — 10.8) 37	4.3 ± 2.1 (1.5 — 7.0) 37	73.7 ± 8.8 (60 — 87) 37
Signi- ficance	P < 0.001	P < 0.001	P < 0.001

n: number of piglets.

age. The isoenzyme activities in serum were raised mainly in the MM fraction compared to non-reacting animals, and a slight but not significant increase was also found in the BB fraction in many halothane sensitive pigs. MB isoenzyme activity was not detectable in the pigs with the exception of some of the non-reacting pigs where very low activities were seen (Table 1).

A pronounced age dependence of CK enzyme activity has been observed in swine. The CK activities in serum increase during the rapid growth phase with a peak activity at about 4 months of age (Jørgensen & Hyldgaard-Jensen 1972). Halothane sensitive pigs are reported to have significantly higher S-CK activity than non-reactants when similar breed and ages are compared. Elevated enzyme activity has therefore been suggested as a possible genetic marker for the stress syndrome in pigs (Woolf *et al.* 1970). It is obvious from this study that the difference in S-CK between halothane reactants and non-reactants is mainly related to the elevated CK-MM isoenzyme activity, probably originating from the skeletal muscles. Therefore, it seems reasonable that muscular abnormalities in the stress susceptible pigs are responsible for the elevation of S-CK.

Increased activity of the BB isoenzyme in serum has been observed in malignant hyperthermia (MH) in man. As malignant hyperthermia occurs during halothane anesthesia in halothane sensitive piglets, the slight raise in BB isoenzyme activity in serum may be connected with the predisposition in these pigs for hyperpyrexia. Moreover, Sporn *et al.* (1978) reported significantly higher BB isoenzyme activity in serum of halothane sensitive pigs than in stress resistant animals.

Differences in muscle fiber types between halothane sensitive

relative percentage in serum of halothane sensitive and piglets.

BB		MB		
total	rel. %	total	rel. %	
2.0 ± 2.6 (0.4 — 9.9)	7.8 ± 4.0 (4 — 15)	—	—	Mean ± s Range n
12	12			
1.2 ± 0.5 (0.3 — 2.2)	22.1 ± 8.3 (8 — 43)	0.3 ± 0.1 (0.1 — 0.5)	5.3 ± 1.3 (3 — 9)	Mean ± s Range n
37	37	14	14	

P < 0.001

and non-reactive pigs have been reported (Lindholm *et al.* 1975). The low oxidative fast fiber types are more pronounced in halothane reactants, whereas the oxidative fiber types are dominating in non-reactants. Further studies will deal with CK activity in these muscle fiber types.

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