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INFLUENCE OF NUTRITION ON MALIGNANT HYPERTHERMIA IN PIGS

By

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JØRGENSEN, P. F.: *Influence of nutrition on malignant hyperthermia in pigs.* Acta vet. scand. 1982, *23*, 539—549. — In 2 experiments malignant hyperthermia susceptible Danish Landrace pigs were fed, for 2 or 4 weeks, synthetic diets containing casein as protein source or no protein. Minerals and vitamins were supplied to both groups. The animals were anaesthetized weekly for a maximum of 20 min with a halothane-oxygen mixture.

In the first experiment malignant hyperthermia was equally delayed in both groups. If malignant hyperthermia developed, the appearance was at the end of the anaesthetic period. In the second experiment a deeper anaesthesia was employed. Malignant hyperthermia was delayed in both groups, but most markedly in the protein-deficient animals. Malignant hyperthermia developed faster after return to the original feed.

These results provide evidence for a nutritional influence on the penetrance of malignant hyperthermia susceptibility during halothane anaesthesia in pigs.

pigs; nutrition and malignant hyperthermia; reduced penetrance.

Malignant hyperthermia susceptibility (MHS) is hereditary with an apparently different mode of inheritance in man and pigs. In man a complex inheritance is suggested (*Gronert* 1980) whereas in pigs most investigations support a recessive inheritance (*Jørgensen* 1981). This discrepancy may reflect different causes or different criteria of MHS in the 2 species. In man MHS is mainly judged by in vitro methods (*Gronert* 1980), whereas in pigs a short-term halothane anaesthesia — a halothane test — is the most frequent identification procedure (*Eikelenboom & Minkema* 1974). During halothane anaesthesia most MHS pigs develop malignant hyperthermia (MH) characterized by progressive muscle rigidity, increase in body temperature, and acidosis within a few minutes of anaesthesia, whereas in man a non-rigid type of MH has been recognized as well (*Britt & Kalow* 1970).

In a few MHS pigs MH will not become manifest during a halothane test because of the influence of modifying factors like breed, age, excitement, technique, environmental temperature, and premedication (*Van den Hende et al.* 1976, *Jørgensen* 1981, *Kallweit et al.* 1980, 1981, *Webb* 1981). Such animals show an incomplete penetrance of MHS. In this investigation it is investigated to what extent MH is modified when pigs are fed synthetic diets.

MATERIALS AND METHODS

Animals and their diet

The experiment comprised 12 genetically known MHS pigs (Danish Landrace) from 2 litters sired by the same boar. Two castrated males and 2 females from 1 litter were tested in experiment 1 and 8 females, from the second litter, in experiment 2. The pigs were fed a diet containing 160 g digestible crude protein per kg (Table 1). At 14—15 weeks of age the pigs' reaction to

Table 1. Composition of diets (g/kg).

	Diet A	Diet B	Control
Starch	350	468	Barley (725)
Sucrose	122	240	Soya grits (240)
Casein	242	00	Fat (10)
Lactose	229	234	$CaHPO_4 \cdot 2H_2O$ (11)
Minerals[a]	57	58	$CaCO_3$ (8)
			NaCl (4)
			Minerals[b] (2)

a: Supplying the diet with the following levels of minerals (g/kg diet): $CaHPO_4 \cdot 2H_2O$ 37, $Na_2HPO_4 \cdot 2H_2O$ 5.2, NaCl 5.0, $CaCO_3$ 3.3, hydrated basic magnesium carbonate (40.0—45.0 % MgO) 3.1, KCl 2.5, $ZnSO_4 \cdot 7H_2O$ 0.52, $FeSO_4$ (dried) 0.35, $CuSO_4 \cdot 5H_2O$ 0.184, $MnSO_4 \cdot H_2O$ 0.184.

After preparation of diets A and B, and immediately before feeding, vitamins were added so as to supply to each pig the following levels of vitamins (mg/day): Retinol 1.5, ergocalciferol 0.025, d'l-α-tocopheryl acetate 25, thiamine · HCl 10, riboflavin 4, nicotinamide 50, pyridoxine · HCl 5, pantothenic acid 10, choline · HCl 250.

b: The mineral-vitamin mix contained vitamins A, D, E, riboflavin, vitamin B_{12}, pantothenic acid, Fe, Cu, Mn, Zn, Co, J, Se at levels equal to or above those recommended by *The Agricultural Research Council* (1981).

Daily supply of feed mixtures to groups A, B and control at 20 kg bodyweight (g/day) was, respectively: 785, 770, 1000.

halothane was confirmed, and the diet was gradually changed. In each experiment 2 pigs, referred to as group A, were fed a synthetic diet containing casein as protein source. Two other pigs (group B) were fed the same diet except that casein was replaced by potato flour and sucrose. In experiment 2 the remaining pigs (groups C and D), were fed the original feed. After 2 weeks of pure experimental feeding in experiment 1 and 4 weeks in experiment 2 the pigs were returned to the original feed. The animals were housed in individual pens at a temperature of approximately 18°C and fed twice a day according to body weight. Water was supplied ad libitum.

Halothane anaesthesia

Apart from group D in experiment 2, all pigs were anaesthetized weekly after 16 h of starvation. After at least 5—10 min rest, anaesthesia was induced and maintained with a halothane-oxygen mixture delivered from a calibrated Fluotec Mk III vaporizer via a face mask in a semi-open system. In experiment 1 induction with 5 % halothane (flow rate 3 l/min) for 2—3 min was followed by maintenance of the anaesthesia with 3—4 % halothane (flow rate 2.5 l/min). In experiment 2 halothane (5 %) was administered for 2 min at a flow rate of 4 l/min and for 1 min at 3 l/min. The anaesthesia was continued with 4 % halothane (flow rate 3 l/min). In both experiments the maximum duration of anaesthesia was 20 min. Anaesthesia was carried out at an environmental temperature of 18—20°C. During anaesthesia the m. biceps femoris temperature was monitored continuously. Malignant hyperthermia was diagnosed on the basis of muscle rigidity leading to dyspnoea and apnoea, increase in the m. biceps femoris temperature, circulatory disturbances in the skin, and confirmed by meaurement of the acid-base status in venous blood. The time of onset of MH was taken to be the time when progressive muscle rigidity developed, the m. biceps femoris temperature increase being taken into consideration.

Statistical analysis

Results from groups A, B, and C in experiment 2 were transformed to logarithmic values and subjected to a two-way analysis of variance including main effects and interaction. Differences between mean values of individual cells were tested according to the a priori method of *Sokal & Rohlf* (1969).

RESULTS

In both experiments growth stopped in the protein-deficient pigs. In experiment 2 where control animals were included, the growth rate of casein-fed pigs was not significantly different from the control animals (Fig. 1). Growth rate of all pigs was low.

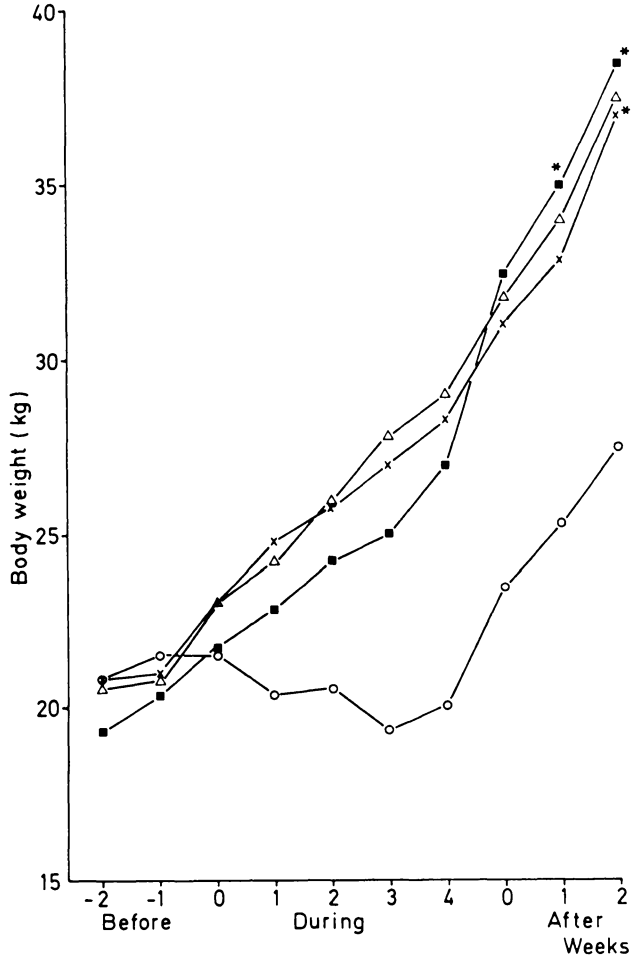

Figure 1. Bodyweight before, during, and after feeding malignant hyperthermia susceptible (MHS) pigs a casein-containing (group A ■——■) or a protein-deficient (group B ○——○) diet. Groups C (×- - -×) and D (△——△) refer to MHS pigs fed a diet containing 160 g crude protein per kg and halothane anaesthetized once weekly (group C) or at the beginning and at the end of the experiment (group D). Two pigs in each group. * indicates that one pig in each group was alive.

Table 2. Results of experiment 1. Duration of anaesthesia (min) before appearance of malignant hyperthermia in pigs before, during and after a period when the pigs were fed a diet supplied with casein (group A) or a protein-deficient (group B) diet. Mean values are geometric means.

		Weeks						
			During			After		
Group	Pig No.	Before —1	0	1	2	0	1	2
A	688	2.0	4.5	20.0+	20.0+	20.0+	7.0	3.5
	689	2.0	20.0+	19.5	20.0+	7.0	3.5	9.0
	Mean	2.0	9.5	19.7	20.0+	11.8	4.9	5.6
B	687	4.0	13.5	20.0+	6.5	20.0	11.0	15.0
	690	1.5	10.0	20.0+	20.0+	20.0+	14.0	10.0
	Mean	2.4	11.6	20.0+	11.4	20.0	12.4	12.2

+ Malignant hyperthermia not developed.

The results of experiment 1 are shown in Table 2. Initially MH appeared within 4 min of anaesthesia. After the change in diet the development was prolonged, and in several cases MH did not occur during the 20 min anaesthetic period. Supply of casein or protein-deficiency did not influence the onset. After return to the original feed, however, MH was, on an average, delayed in the protein-deficient pigs compared to the casein-fed animals where pre-experimental results were approached.

In experiment 2 MH initially occurred within 3 min of anaesthesia (Table 3). During the period of experimental feeding a significant delay in the onset was observed in casein-fed and protein-deficient pigs compared with as well the initial values as those of control fed pigs tested the same day. Towards the end of the experiment protein-deficient animals reacted slowest. After return to the original feed the initiation of MH in both groups approached initial values. The control animals tested weekly (group C) presented a slight, but significant delay during the last 2 weeks of the experimental period. Control animals tested only before and after the experiment (group D) reacted fast during all of the 3 anaesthetic periods.

Towards the end of experiment 2 three pigs died of MH as a direct consequence of the anaesthesia. The pigs weighed between 30 and 35 kg.

Table 3. Results of experiment 2. Duration of anaesthesia (min) before appearance of malignant hyperthermia in pigs before, during and after a period when the pigs were fed a diet supplied with casein (group A) or a protein-deficient (group B) diet. Groups C and D were fed a diet containing 160 g digestible crude protein per kg. Mean values are geometric means.

Group	Pig No.	Weeks: Before		During					After			
		—2	—1	0	1	2	3	4	0	1	2	3
A	697	1.5	1.5	3.0	11.0	9.0	4.5	10.0	8.5	6.0	9.5	3.5
	700	1.5	3.0	4.0	4.5	8.3	13.5	7.5	6.0	4.3†	—	—
	Mean	1.5	2.1	3.5	$7.0^{a,***}$	$8.6^{b,***}$	7.8^{***}	8.7^{***}	7.1^{***}	5.0^{**}	—	—
B	694	2.5	2.0	1.5	3.0	7.0	10.0	16.0	19.0	9.5	6.0	—
	696	2.5	1.5	3.0	4.5	8.0	16.0	17.0	11.5	2.5	2.0	—
	Mean	2.5	1.7	2.1	3.7	$7.5^{a,***}$	$12.6^{b,***}$	$16.5^{b,***}$	$14.8^{c,***}$	4.9^{**}	3.5	—
C	693	2.5	1.5	2.5	2.0	2.3	3.3	6.0	4.0	3.0	3.0†	—
	698	2.5	1.5	2.0	2.8	3.0	4.5	5.0	3.0	2.8	2.5	—
	Mean	2.5	1.5	2.2	2.3	2.6	3.8	5.5	3.5	2.9	2.7	—
D	695	2.5	1.5	—	—	—	—	—	—	—	—	1.5†
	701	1.0	1.5	—	—	—	—	—	—	—	—	1.5

† Died of malignant hyperthermia in connection with anaesthesia.
A, B or C different from common pre-experiment value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
A or B different from C on the same date: a $P < 0.05$, b $P < 0.01$, c $P < 0.001$.

DISCUSSION

It is difficult to measure the time of appearance of the first clinical symptoms of malignant hyperthermia precisely. The method used in this experiment only gives an approximation of this time. The rapidity of onset may also be estimated as the anaesthetic time necessary to produce an increase in body temperature of 0.5°C in MHS pigs. By using this criterion the same results were reached. In especially fast reacting animals, however, such increase may have occurred before the temperature can be measured with reliability, and to wait for a further 0.5°C temperature increase before interruption of anaesthesia, involves a very high risk of a lethal course.

In spite of such difficulties in assessing the first symptoms, the experiments clearly demonstrated a modified expression of

MH in pigs fed synthetic diets as onset was delayed, in some situations beyond 20 min. In experiment 1 a more pronounced delay was observed than in experiment 2. This difference may be caused by the use of a deeper anaesthesia in experiment 2 and by individual variability in the response to the dietary changes. All pigs would, however, have been classified as non-reactors with respect to MH, if a short-term halothane anaesthesia (3—5 min) had been used. In addition to the delay in the onset of MH the clinical symptoms were modified. Especially in the protein-deficient pigs, muscle rigidity became less manifest, making the syndrome resemble non-rigid MH in man (*Britt & Kalow* 1970).

The halothane test should be used in pigs older than 6—8 weeks, as younger MHS pigs frequently are classified as non-reactors (*Jørgensen* 1981, *Webb* 1981). In older pigs a standardized halothane test results in an increase of the average anaesthetic time necessary to induce MH, a decrease in the frequency of reactors, and an increase in doubtful reactors, if the maximum duration of anaesthesia is 5 min (*Kallweit et al.* 1980). In the present investigation, reaction time of control pigs anaesthetized weekly increased from initially 2.0 min to 2.8 min and remained unchanged in control pigs anaesthetized only at the beginning and the end of the experiment. Consequently an age effect was present in repeatedly tested pigs, but such an effect cannot explain the findings in the pigs fed synthetic diets. Environmental influences on the MH reaction (*Kallweit et al.* 1981) can also be excluded, as all animals were housed and treated in the same way.

According to *Mabry et al.* (1981) adequate energy stores in striated muscles are probably necessary to initiate and maintain MH. In the present investigation all pigs were fed the same energy levels, making such an explanation of a modification of MH unlikely. *Marchsteiner et al.* (1978) have noted that the manifestation of MH in pigs may be positively or negatively influenced by purposeful feeding, without defining this term more specifically.

Protein levels and amino acid composition in the feed influence protein synthesis and levels of protein and free amino acids in muscle tissue of several species (*Wannemacher & Allison* 1968). In some tissues specific amino acids modify intra-

cellular calcium metabolism (*Wassermann & Taylor* 1969, *Wollheim & Sharp* 1981) which is considered to be of major importance for MHS (*Gronert* 1980). The findings in the present investigation might be a consequence of an altered intracellular amino acid metabolism caused by quantitative and qualitative differences in the amino acid composition of the diets.

Diets A and B contained less lipids (4.8 g/kg and 1.3 g/kg) than the control diet (36.9 g/kg). Protein-free and low-level protein diets with a low lipid content partially protect rats against carbon tetrachloride poisoning and lead to reduced activities of microsomal drug-metabolizing liver enzymes (*McLean & McLean* 1966, *Marshall & McLean* 1971). Whether similar mechanisms are responsible for the findings in this investigation is uncertain.

Potassium influences intracellular protein and amino acid metabolism (*Arnauld et al.* 1981). Potassium intake was low (1.0—1.2 g/day) in pigs fed the experimental diets compared with control-fed pigs (4.9—5.4 g/day). As a result, plasma levels of potassium were lower in the casein-fed animals (3.6 ± 0.4 mmol/l) compared to protein-deficient (5.3 ± 0.8 mmol/l) and control-fed pigs (5.2 ± 0.4 mmol/l). A moderate potassium deficiency in casein-fed animals (*Aitken* 1976), might contribute to the altered reaction in these pigs.

Magnesium was also supplied in lower amounts to the experimental groups (0.6—0.7 g/day) than to the control animals (1.2—1.3 g/day). Plasma levels of magnesium (0.80—0.99 mmol/l) were within the normal range, and a primary influence of low magnesium intake is not considered to be of importance for the findings.

Selenium, iodine, and vitamin B_{12} was not included in the experimental diets. In view of the short duration of the experiments it is unlikely that a deficiency of these elements should have developed (*Underwood* 1971) since the animals were fed a diet supplemented with selenium, iodine, and vitamin B_{12} from weaning until the start of the experimental feeding. It cannot, however, be excluded that repeated episodes of MH may increase the demand for one or more of the elements.

In conclusion the feed composition may influence MH in MHS pigs. The specific diet component or components responsible for the effects are unknown, and furthermore it remains to be deter-

mined whether such effects are of major importance for reduced penetrance of MHS during halothane anaesthesia under practical conditions. Further investigations are in progress on the influence of different feed components on MH in pigs.

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SAMMENDRAG

Ernæringsbetingede ændringer af malign hyperthermi hos svin.

I to undersøgelser blev malign hyperthermifølsomme Dansk Landrace grise fodret med syntetiske foderblandinger tilsat kasein som proteinkilde eller uden protein. Mineraler og vitaminer blev tilført begge grupper. Dyrene blev anæsteseret hver uge med halothan i maksimalt 20 min.

I første undersøgelse forsinkedes udviklingen af malign hyperthermi lige meget hos begge grupper. Hvis malign hyperthermi udvik-

ledes, skete dette ved slutningen af de 20 min. I anden undersøgelse benyttedes en dybere anæstesi. Malign hyperthermi forsinkedes atter hos begge grupper, mest udtalt hos proteinmanglende dyr. Ved tilbagevenden til udgangsfoderet indtrådte malign hyperthermi hurtigere.

Resultaterne viser, at foderets sammensætning kan influere på penetransen af malign hyperthermifølsomhed hos svin under halothananæstesi.

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