Stress testing effects on blood lipid concentrations and organic lipid composition of different genotypes of swine.

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SUMMARY

An easy and accurate method to determine susceptibility to malignant hyperthermia (MH, i.e. increased stress susceptibility) in swine is still lacking. The physiological evaluation of the response of swine to stress may be a useful mean to accomplish this. Hence, the objective of this study was to investigate the effects of physical stress (i.e. treadmill exercise) or metabolic stress (i.e. an oral glucose load) on blood lipid concentrations and their relations to muscle and liver lipid composition in various swine genotypes. Cholesterol and free fatty acid levels were determined by appropriate methodology. In addition, plasma catecholamine levels were obtained. We report here significant differences in the pre-stress cholesterol levels between the normal and MH susceptible animals as well a several differences in the response of these animals only to the imposed stresses (primarily in catecholamine levels). These data indicate that determining plasma levels of cholesterol and catecholamines under various physiological conditions may be useful in determining susceptibility to MH in swine.

INTRODUCTION

One of the most striking features of animals considered to have malignant hyperthermia (MH) which includes an increased stress susceptibility is their abnormal growth patterns. These animals grow more rapidly, have a hypertrophic musculature and a leaner muscle mass than their genetically normal counterparts. However, the mechanisms underlying these difference remain largely unknown.

It has been suggested for some time that swine susceptible to MH have an altered lipid metabolism. More recently, it was suggested that the genetic defect in MH may be within the gene coding for a hormone sensitive lipase found in muscle (Levitt et al., 1990). However, there is more abundant data suggesting that the primary defect in malignant hyperthermia is within the calcium release channel of the sarcoplasmic reticulum (e.g. Fill et al., 1990, Maclennan et al., 1990, McCarthy et al., 1990, Mickelson et al., 1990). Nevertheless in either case, secondary changes within skeletal muscles are likely which will be related to or underlie the formentioned altered growth patterns in MH animals. For example, our laboratory has recently shown that the phospholipid compositions and fatty acid profiles of both cardiac and skeletal muscles are altered in MH animals (Seewald et al., 1991). In was suggested from these findings that these differences alter the structural stability of muscle membranes which will play a role in the proliferation of a MH reaction. This was predicted to be the case whether or not the stress to the animal was environmentally (e.g. elevated temperature) or chemically (e.g. via halothane) induced.

We hypothesize here that MH susceptible animals which elicit a lower tolerance to stress will have a decreased ability to regulate their energy metabolism when faced with a stress. As a means to quantitate such behavior we determined changes in the concentrations of lipids within blood following either physical (treadmill exercise) or metabolic stress (oral glucose load). In addition, these levels were compared to those found in various organs (including liver and muscle). Finally, all measurement were compared between swine with various genetic backgrounds related to MH susceptibility.

MATERIALS AND METHODS

Three groups of German landrace swine were studied: homoyzgous halothane negative animals (H-/H-; n = 3); heterozgous negative animals (H-/h +; n = 6) and homozygous halothane positive (h + /h +; n = 3) animals. The genetic background of these animals was derived from the special breeding program at our institute, whereas halothane sensitivitiy was determined using the branyard halothane challenge. At an average body weight of 116 kg each animal was investigated. Two sets of control blood samples were obtained 12 h after fasting. Immediately following treadmill exercise for 20 minutes additional blood samples were removed. Two exercise experiments were performed, each comprising 20 min. treadmill work (E1, E2). Several days later the same animals were exposed to a glucose tolerance test (i.e. oral glucose 2 g/kg) and one hour later the final set of samples obtained. Total plasma cholesterol and free fatty acid (FFA) levels were measured enzymatically. Plasma catecholamine levels were determined by HPLC analysis (Boos et al., 1987).

All animals were slaughtered at an average body weight of 127 kg (approximately 1 week post stress responses) and samples of liver, the longissimus dorsi muscles and the supraspinatus muscles removed. Total lipid concentrations were determined from each sample as previously reported (Seewald and Eichinger, 1989). The samples were then saponificated and the total cholesterol was measured colormetrically as ironoxide-complexes (Mann, 1961; Tu et al., 1967).

Statistical significance was determined by ANOVA.

RESULTS AND DISCUSSION

Control levels of total plasma cholesterol were significantly greater in the homoyzgous negative, H-/H-, animals compared to the MH positive animals, H-/h + and h + /h + (Tab. 1). In contrast, epinephrine and norepinephrine levels were higher in the positive animals. No differences in the plasma free fatty acids were observed between groups (Tab. 1).

Exercise had no effect on the plasma levels of cholesterol nor FFAs, but resulted in increased catecholamine levels in all animals (Tab. 1). The increases in both epinephrine and norepinephrine were most pronounced in the h + /h + animals relative to the other two groups ($p < 0.05$).

The oral administration of glucose significantly enhances the levels of total plasma cholesterol in all animals ($p < 0.05$). However, the relative increases were the same between the groups. FFAs levels were unchanged in all animals, whereas with exercise the catecholamine levels were most dramatically increased in the h+/h+ animals (Tab. 1)

Table 2 provides the post-mortem levels of cholesterol determined for each genotype in each of the three samples (i.e., longissimus dorsi and supraspinatus muscles, and the liver). For each sample type, the cholesterol levels were greater in the h+/h+ animals followed by the H-/h+ groups with the lowest levels found in the normal animals H-/H-. However, these differences did not reach significance perhaps due to the small group sizes.

CONCLUSIONS

Although these results are preliminary, they do suggest that the cholesterol membrane component and perhaps cholesterol metabolism in general differs between the animals susceptible to MH (h+/h+ and H-/h+) and those which are normal (H-/H-). In addition, only in those animals which are highly sensitive to the in vivo administration of halothane (h+/h+) is the release of catecholamines following some sort of stress exaggerated. Perhaps simple physiological tests can be designed using this information that will lead to a new, more accurate, but simple assessment of MH susceptibility in swine.

Tab.1: Contents of cholesterol (Chol.), free fatty acids (FFA) epinephrine (E) and norepinephrine (NE) prior to stress (N), after running on a treadmill (E1 and E2) and after application of 2g glucose-monohydrate/kg BM (GTT), obtained from three genotypes of swine.

		N			E1			E2			GTT		
		h+/h+ n=3	h+/H- n=6	H-/H- n=3	h+/h+ n=3	h+/H- n=6	H-/H- n=3	h+/h+ n=3	h+/H- n=6	H-/H- n=3	h+/h+ n=3	h+/H- n=6	H-/H- n=3
Chol. (mg%)	LSM	82.32	75.61	108.90	83.90	75.42	105.01	-	-	-	99.80	83.86	126.28
	STD	4.47	3.41	6.57	6.32	4.65	8.55				6.32	4.65	8.55
FFA (mmol/l)	LSM	0.48	0.55	0.46	0.55	0.67	0.67	0.63	0.90	0.73	0.37	0.36	0.46
	STD	0.06	0.05	0.09	0.08	0.06	0.11	0.09	0.06	0.11	0.10	0.06	0.11
E (nmol/l)	LSM	2.09	0.89	1.42	3.62	1.13	3.06	15.12	1.50	3.01	5.09	1.18	2.29
	STD	1.87	1.41	2.67	2.56	1.96	3.52	2.64	1.93	3.52	2.65	1.93	3.52
NE (nmol/l)	LSM	6.22	4.92	3.38	9.19	7.93	7.87	24.76	4.51	1.67	23.56	9.44	1.52
	STD	2.84	2.13	4.04	4.02	2.93	5.33	4.01	2.93	5.33	4.01	2.93	5.33

LSM = least square mean values; STD = standard deviations
h+/h+ = halothane positive, malignant hyperthermia susceptible
h+/H- = halothane negative, malignant hyperthermia susceptible
H-/H- = halothane negative, normal

Tab.2: Cholesterol content in two muscles (m. long. dorsi = MLD, m. supraspinatus = MSP), liver and erythrocytes from three genotypes of swine (mg%).

	CHOLESTEROL LEVELS (mg%)				
	pooled data n = 12	h+/h+ n = 3 (a)	h+/H- n = 6 (b)	H-/H- n = 3 (c)	t-test
MLD (wet weight)	41.03 (7.8)	44.19	39.34	38.30	-
MSP (wet weight)	47.89 (8.3)	54.62	46.17	44.20	a:b * a:c *
liver (wet weight)	276.06 (46.2)	297.74	287.37	276.85	-
erythrocytes (dry matter)	396.81 (21.7)	417.28	386.70	396.56	-

pooled data: mean values, () = standard deviations
* = signifikant differences between groups, $p < 0.05$
h+/h+ = halothane positive, malignant hyperthermia susceptible
h+/H- = halothane negative, malignant hyperthermia susceptible
H-/H- = halothane negative, normal

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