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Phospholipid- and fatty acid pattern influenced by genetic and nutritional factors in swine heart muscle

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SUMMARY

Stress susceptability in swine is known to be associated with an abnormal intracellular calcium regulation (1) related to functional disorders of the intracellular membranal systems (2,3) which caused these muscles to be extremely sensitive to halothane reactions. Thirteen homozygous halothane positive (h^-/h^-), 12 homozygous halothane negative animals (H^+/H^+) and 14 heterozygous negative animals (H^+/h^-) were used in this experiment, where additionally a selected 18 of these animals were fed a diet rich in n-3 fatty acids. Post mortem heart muscle was analysed for phospholipid and fatty acid composition using HPLC and GC techniques (4).

All halothane positive animals showed significant higher amounts of lyso-phosphatidylethanolamine (LPE) than the homozygous halothane negative animals. Likewise, the most prominent phospholipid fractions differed significantly in their fatty acid pattern between the three genotypes. Supplementation with n-3 fatty acids caused significant increases in these specific fatty acids within phosphatidylcholine (PC) in the halothane negative animals, but not in the homozygous halothane positive animals. Our results indicate significant variations of phospholipid and fatty acid patterns in the heart muscle of swine with different stress susceptability and also that these defined animal groups react differently to a n-3 fatty acid supplementation.

INTRODUCTION

Phospholipids are an integral part of the lipid matrix which form the basic structures of cell membranes; their distribution and fatty acid patterns are also crucial for transmembranal signaling and for other intracellular functions (5,6). Swine from different breeding population known to be susceptible to malignant hyperthermia were recently shown to differ in their phospholipid and fatty acid composition in both skeletal and cardiac muscle membranes compared to normal controls (7). According to empirical studies, n-3 fatty acids (e.g. eicosapentaenoic acid (EPA), C 20:5; docosahexaenoic acid (DHA), C 22:6) are considered to have protective effects against cardiovascular diseases. Thus, it was the aim of this study, to again investigate the variation of the phospholipid and fatty acid pattern in the heart muscle of swine with different stress susceptability, but also to study the effects of diet, normal and high in n-3 fatty acids, on the ultimate lipid composition of heart muscle.

MATERIAL and METHODS

Thirtynine male castrated German Landrace pigs were obtained from special breeding programs and tested for sensitivity to halothane (barnyard challenge) and for creatine kinase activity in plasma. Three genotypic groups were defined:

- homozygous halothane positive swine (h^-/h^-), n = 13;
- heterozygous halothane negative swine (H^+/h^-), n = 14; and
- homozygous halothane negative swine (H^+/H^+), n = 12.

The animals were also divided into two feeding groups, one received a diet with 5% fishoil rich in n-3 fatty acids, the other received a counterbalanced diet with 5% coconut fat. They were fed the same diet over the whole fattening period.
Immediately after slaughter ventricular heart muscle was removed, homogenized and lipids extracted by Chloroform/Methanol (8). Phospholipids were separated by HPLC techniques and subsequently the fatty acids transesterified to fatty acid methyl esters

and further separated by gaschromatography (4).
Data were evaluated by analysis of variance using the following model: y = μ + genotype + feeding group + genotype*feeding group + e.

RESULTS and DISCUSSION

The amount of lipid in heart muscle was similar in all animals. In general, 2.77±0.19% ($\bar{x}$−S.D.) of the total wet weight of the heart was lipid, and 84.1±8.7% of this component was phospholipids.
In contrast, the specific phospholipid composition was quite varied between genotypes (Tab.1). Halothane positive swine (h^-/h^-) were found to have significantly higher amounts of lyso-phosphatidyl-ethanolamine (LPE) in their pattern relative to the homozygous halothane negative swine (H^-/H^-); this difference was partly compensated by a lower phosphatidyl-ethanolamine (PE) content in the positive animals.
The relative amount of linoleic acid (C 18:2) was significantly increased in PE, LPE and phosphatidyl-choline (PC) in h^+/h^- and H^-/h^+ animals compared to the normal or H^-/H^- type (Tab.2). Some decreases in arachidonic acid (AA; C 20:4) were noted in the h^+/h^+ and H^-/h^+ animals; these changes were not significant, but consistant with our previous findings (7).
In general, n-3 fatty acid supplementation enhanced the relative amounts of EPA and DHA in cardiac phospholipids and caused the amount of AA to be reduced (Tab.3).
However, what was most interesting, was that this was not true for the halothane positive animals, which have a PC composition with unchanged amounts of n-3 fatty acids between the two feeding groups (Tab.4).

Tab.1: Phospholipid composition of swine cardiac muscle in different genotypes (μmol/g neutral fat free wet muscle weight); least square means

phospholipid fraction	pooled data	genotype a H^-/H^- n=12	genotype b H^-/h^+ n=14	genotype c h^+/h^+ n=13	sign.
cardiolipin	1.74 (1.52)	1.20	1.49	2.08	n.s.
p-inositol	0.65 (1.31)	0.11	0.39	0.98	n.s.
p-serine	0.76 (1.37)	0.56	0.61	0.65	n.s.
p-ethanolamine	2.14 (2.37)	2.72	2.01	1.82	n.s.
l-p-ethanolamine	3.12 (1.94)	1.73	2.82	3.56	a:c *
p-choline	3.40 (1.30)	2.83	3.49	3.20	n.s.
l-p-choline	1.33 (2.11)	1.29	0.85	0.93	n.s.
sphingomyelin	0.85 (0.58)	0.82	0.82	0.87	n.s.

pooled data: mean values; () = standard deviations
H^-/H^- = homozygous halothane negative
H^-/h^+ = heterozygous halothane negative
h^+/h^+ = homozygous halothane positive
p- = phosphatidyl;
l-p- = lyso-phosphatidyl

a:c = significant difference ($p<0.05$) between H^-/H^- and h^+/h^+ animals
n.s.= no significant difference between any possible group comparison.

Tab.2: Relative amounts of linoleic and of arachidonic fatty acids in three phospholipid fractions in swine cardiac muscle within the three defined genotypes (% of all phospholipid bound fatty acids); least square means

phospholipid fraction	genotype a H^-/H^- n=12	genotype b H^-/h^+ n=14	genotype c h^+/h^+ n=13	sign.
p-ethanolamine				
C 18:2	11.7	15.0	15.6	a:b * a:c **
C 20:4	12.0	11.4	11.5	n.s.
l-p-ethanolamine				
C 18:2	15.3	19.7	18.6	a:b *
C 20:4	13.4	11.5	10.8	n.s.
p-choline				
C 18:2	19.7	22.6	23.0	a:b * a:c *
C 20:4	2.7	2.7	2.8	n.s.

H^-/H^- = homozygous halothane negative
H^-/h^+ = heterozygous halothane negative
h^+/h^+ = homozygous halothane positive
C 18:2 = linoleic acid, C 20:4 = arachidonic acid
p- = phosphatidyl, l-p- = lyso-phosphatidyl
* = signif. diff. $p<0.05$ ** = signif. diff. $p<0.01$
n.s.= no significant difference between any possible group comparison.

Tab.3: n-3 and arachidonic fatty acid amount in lyso-phosphatidyl-ethanolamine in swine cardiac muscle following fishoil (n-3) or counterbalanced diet with coconut oil supplementation (nmol/g neutral fat free wet muscle weight); least square means

fatty acid	pooled data	dietary supplem. fishoil	dietary supplem. coconut	diff.	sign.
C 20:4	322.3 (168.4)	225.2	369.8	-144.6	*
C 20:5 n-3	440.6 (199.3)	515.2	290.2	+225.0	**
C 22:6 n-3	195.5 (93.1)	219.3	125.7	+ 93.6	**
total n-3 fatty acids	641.9 (293.5)	734.8	423.3	+311.5	**

pooled data: mean values; () = standard deviations
C 20:4 = arachidonic acid, C 20:5 = eicosapentaenoic acid,
C 22:6 = docosahexaenoic acid
* = signif. diff. $p<0.05$
** = signif. diff. $p<0.01$

Tab.4: Effects of supplementation of n-3 fatty acids (fishoil) on the fatty acid pattern of phosphatidylcholine (PC) in different MHS-genotypes (% fatty acid of all PC bound fatty acids)

	genotype					
	H^-/H^-		H^-/h^+		h^+/h^+	
	fish	coco	fish	coco	fish	coco
	a	b	c	d	e	f
n	6	6	7	7	5	7
C 20:5 (n-3)	5.1	1.9	4.9	2.1	3.9	3.3
sign. between groups	a:b ***		c:d ***		n.s.	
C 22:6 (n-3)	1.9	0.0	2.1	0.3	0.9	1.1
sign. between groups	a:b ***		c:d ***		n.s.	
total n-3 fa	7.2	2.2	7.2	2.5	4.8	5.3
sign. between groups	a:b ***		c:d ***		n.s.	

least square means
H^-/H^- = homozygous halothane negative
H^-/h^+ = heterozygous halothane negative
h^+/h^+ = homozygous halothane positive
fish = fish oil supplementation coco = counterbalanced with 5% coconut oil
total n-3 fa = total n-3 fatty acids
*** = signif. diff. $p<0.001$
n.s.= no significant difference between feeding groups.

CONCLUSIONS

Phospholipids are the major components of the total lipid composition of the heart and were found in distinctive pattern (i.e. amount of each type of phospholipid) in each genotype. For example, lyso-phosphatidylethanolamine content was significantly increased in the halothane positive swine. This may be indicative for an increased content of alkyl and alkenyl analogues. The halothane positive animals also did not incorporate dietary supplemented n-3 fatty acids in PC, whereas the halothane negative pigs did so in significant amount. These data may indicate that swine susceptible to malignant hyperthermia not only have different membrane compositions, but also have altered mechanisms for lipid metabolism.

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