Effects of a high n–3 fatty acid diet on membrane lipid composition of heart and skeletal muscle in normal swine and in swine with the genetic mutation for malignant hyperthermia

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Abstract. Knowledge concerning the genetic defects underlying malignant hyperthermia (MH) has expanded rapidly in recent years. In contrast, our understanding of the accompanying physiological changes is less clear. In this regard, the aim of this study was to determine whether normal swine and swine susceptible to MH (both heterozygous and homozygous animals) differ in their abilities to incorporate n–3 (omega 3) fatty acids into their skeletal and heart muscles. Swine of each genotype were fed either a diet rich in n–3 fatty acids (i.e., 5% fish oil) or an equal caloric diet low in n–3 fatty acids (i.e., 5% coconut oil). All dietary supplementations were given over a 13-week period. Subsequently, for each muscle type the following was determined: *1*) the relative fatty acid profiles of eight different phospholipid classes and of neutral lipids, and *2*) the total phospholipid and the total lipid content. The incorporation of n–3 fatty acids (i.e., eicosapentaenoic acid and docosahexaenoic acid) occurred within the various phospholipids and neutral lipids without influencing their total lipid content. The increased content of n–3 fatty acids in neutral lipids of skeletal muscle was related to a decreased content of medium-chain saturated fatty acids, whereas an increased incorporation of n–3 fatty acids into the membrane phospholipids was often related to a decreased content of linoleic acid and/or arachidonic acid. In general, the pattern of n–3 fatty acid incorporation was considerably different between the normal animals and the MH homozygous and heterozygous animals. The significant interaction between diet-induced n–3 fatty acid profiles and the stress-susceptible MH genotype may indicate an altered mechanism for fatty acid turnover and a repair mechanism to maintain cellular functions and structure.—**Otten, W., P. A. Iaizzo, and H. M. Eichinger.** Effects of a high n–3 fatty acid diet on membrane lipid composition of heart and skeletal muscle in normal swine and in swine with the genetic mutation for malignant hyperthermia. *J. Lipid Res.* 1997. **38:** 2023–2034.

Supplementary key words diet supplementation • fish oil • eicosapentaenoic acid • docosahexaenoic acid • phospholipids

Recent findings concerning the molecular genetics of malignant hyperthermia in humans have indicated that this syndrome has heterogenic origins (1–7), whereas in swine susceptible to malignant hyperthermia (MH) a single point mutation is considered to cause this disorder in all strains in which this syndrome has been found (8, 9). In swine, a single base pair substitution (A614C) within the gene for the skeletal muscle sarcoplasmic reticulum calcium release channel (i.e., the ryanodine receptor, RYR1) is considered to underlie susceptibility to MH (2, 8, 10). This discovery has made it possible to develop a diagnostic test for normal, heterozygous, and homozygous carriers of the mutation in all breeds of swine, allowing breeders to eliminate the gene mutation from their herds (8).

One of the dramatic signs of an acute malignant hyperthermia episode is the development of sustained force by skeletal muscle. These contractures can also be initiated in vitro and are well correlated with increases in intracellular [Ca^{2+}] (11–13). Increases in intracellular [Ca^{2+}] result in sustained force production (contracture), substrate (ATP) depletion, grossly increased oxygen consumption, increased production of carbon dioxide, and the build-up of vast quantities of metabolic acids (11). Eventually, membrane function is disrupted with release of metabolites, potassium, and myoglobin into the circulation. Heat production by the large muscle mass (>50% of total body weight in humans) in a state of contracture increased the central body ('core') temperature (14–16).

Although the abnormal regulation of intracellular [Ca^{2+}] in malignant hyperthermia is considered as the alteration of greatest relevance leading to a MH epi-

Abbreviations: MH, malignant hyperthermia.

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sode, numerous other alterations within skeletal muscle have been described (17–23). It is not known how these, perhaps secondary alterations, may contribute to the overall effects of MH and/or other phenotypic features of susceptible animals, but such parameters need consideration. One of the well-known phenotypic characteristics associated with MH, in swine, is skeletal muscle hypertrophy (24–26). Heavy muscling, altered energy metabolism, and hypermetabolism during MH episodes are only a few of the physiological alterations associated with the MH genotype (27). However, the underlying cause of these features is not known, but it was previously reported that swine with different MH genotypes differ in their phospholipid and fatty acid composition in skeletal and cardiac muscle membranes (28). These differences in composition may further suggest an underlying difference in muscle metabolism that needs further clarification.

Omega 3 (n–3) fatty acids are important precursors of eicosanoids that have different physiological effects compared to those derived from n–6 fatty acids (29–35). N–3 fatty acids can be incorporated into biomembranes and influence cellular structure (e.g., fluidity) and function (e.g., intracellular second messenger mechanisms) (36–40). It has been shown that it is possible to incorporate these fatty acids into the various membrane phospholipids in swine (41). However, it is not known whether this ability might be different in swine with different susceptibilities to MH. Therefore, in the present study, we compared the fatty acid patterns in membrane lipids of skeletal and heart muscle of swine that were fed a diet high in n–3 fatty acids to the patterns in animals fed a diet low in n–3 fatty acids. In addition, the influence of the MH genotype of the animals on the individual fatty acid pattern was determined.

MATERIAL AND METHODS

Animals

Thirty-nine male, castrated German Landrace swine were obtained from different breeding schemes, which were selected either for MH homozygous or MH-free animals (normal animals). MH heterozygous animals were established by crossbreeding MH homozygous and normal animals. All animals were tested for their sensitivity to halothane (barnyard challenge test). Additional information was achieved by gene markers, which were linked to the MH gene. Thirteen of these animals were homozygous and 14 animals were heterozygous for the MH locus. Twelve animals were normal MH-free animals.

Fatty acid supplementation

These animals were further divided in two feeding groups. One group was fed a diet supplemented with 5% fish oil (red tobis oil), which is rich in n–3 fatty acids (i.e., eicosapentaenoic acid, C20:5 n–3; docosahexaenoic acid, C22:6 n–3). The other group served as a control group that was fed a diet low in n–3 fatty acids (i.e., 5% coconut oil). Both nonpurified diets had comparable nutritional value (8.4% total fat, 17.9% total protein with a 14.0% megajoule metabolizable energy) and were fed as pellets. The fish oil contained 350 ppm ethoxyquin as an antioxidant and the diets were additionally enriched with 100 mg α-tocopherol and 500 μg selenium per kg feed to prevent the formation of oxidation products. Samples were taken from every feed bag, pooled and the fatty acid patterns of each diet were analyzed twice by gas chromatography according to the method described below. The fatty acid patterns of the two diets are shown in **Table 1.** Feeders were cleaned daily and fresh food was given twice a day. All supplementations were given over a 13-week period, which was initiated when the animals had an average weight of 29 kg (i.e., at approximately 12 weeks of age).

The animals were slaughtered at a weight of approximately 100 kg. Immediately after electrical stunning and bleeding, samples of cardiac muscle (left ventricle)

TABLE 1. Individual fatty acid composition (% of total fatty acids) of two different diets, fish oil rich in n–3 fatty acids and control diet of coconut oil

Fatty Acids	Diet Type: Fish Oil High n–3	Diet Type: Coconut Oil Control
	% total fatty acids	
C12:0	1.0	34.8
C14:0	5.4	12.9
C16:0	21.7	15.8
C16:1	5.8	1.0
C18:0	4.1	5.1
C18:1 *cis*	14.0	14.6
C18:2n–6	14.3	9.0
C18:3n–3	3.0	1.2
C18:4n–3	4.8	0.4
C20:1	1.8	0.4
C20:4n–6	0.9	0.0
C20:5n–3	10.1	0.9
C24:0	0.7	0.2
C22:6n–3	10.7	1.0
Subgroups		
Total n–3	28.6	3.5
Total n–6	15.2	9.0
Polyenic	43.8	12.5
Monoenic	22.2	15.9
Saturated	34.0	71.6
Polyenic/Saturated	1.3	0.17

Data represent mean values from a duplicate analysis of pooled feed samples.

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and supraspinatus muscle were obtained, stored in a plastic bag, vacuumized and frozen at −30°C until further analysis. Animals from both supplementation groups did not differ significantly in daily gain, body weight, heart weight, carcass composition, or meat quality criteria.

Methods

The total lipids within the tissue samples were extracted by the method described by Bligh and Dyer (42) with chloroform–methanol 1:1 by volume. The major phospholipid classes, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, cardiolipin, sphingomyelin, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, and the neutral lipids in the total lipid extracts were separated by high pressure liquid chromatography and collected using a fraction collector according to the procedure of Seewald and Eichinger (43). The solvent system for elution required the simultaneous use of pH and polarity gradients. The system consisted of the following progession of solvent changes: *1*) elution with acetonitrile for 5 min; *2*) elution with acetonitrile containing 0.2% phosphoric acid which was changed to methanol containing 0.2% phosphoric acid between 15–35 min. The flow rate was maintained at 1 ml/min and the detection wavelength was 205 nm. The lyso-phosphatidylcholine and lyso-phosphatidylethanolamine fractions also represent the plasmalogen content of the respective phospholipid. This was shown by a gradient system at pH 6.8, where lysoproducts were detected only after treatment with concentrated HCl fumes (43). The lysis of the ether binding is a result of the low pH of the mobile phase used in this method (28, 43).

The individual fatty acids in the lipid fractions were transesterified to fatty acid methyl esters as described by Shehata, De Man, and Alexander (44). Using this procedure, transesterification of fatty acids was nearly 100% as determined by thin-layer chromatography (28). The fatty acid methyl ester concentrations were determined using a gas chromatograph (HP 5790A; Hewlett-Packard, Bad Homburg, Germany) equipped with a capillary column (30 m × 0.25 mm, DB-23; J&W, Folsum, CA). The carrier gas was H_2 and the flow rate was 2 ml/min. with a split of 1:20. The chromatograph temperature started at 170°C and was increased 4.5°C/min. until a temperature of 220°C was obtained, at which it was held for 10 min. Using this method, the relative concentrations of 29 different fatty acid species were determined for each sample with nonadecanoic acid, C19:0, as an internal standard.

The individual phospholipid concentrations were calculated after the amount of fatty acids found in each fraction (43). The amount of neutral lipids was calculated on the supposition that all fatty acids found were derived from triglycerides. Neutral lipid and phospholipid concentrations were described as µmol phospholipid/g wet muscle weight.

Statistical analysis

Data were analyzed by analysis of variance using the general linear model procedure of SAS® (45). The statistical model for the analysis of total lipid content and phospholipid composition included MH genotype and supplementation group as main effects, the two-fold interaction between these effects and body weight as a covariate. The statistical model used for the analysis of the individual fatty acid patterns included the total lipid content as an additional covariate.

RESULTS

Total lipid content, neutral lipid content, and membrane phospholipid composition

The total lipid, the neutral lipid, and the total phospholipid content in both tissues did not differ significantly among the genotypes and supplementation groups examined (**Table 2** and **Table 3.**) A significant influence in the individual phospholipid composition was found for the interaction between genotype and supplementation group for the amount of phosphatidylcholine in the cardiac muscle. MH heterozygous animals fed a diet supplemented with fish oil showed higher amounts of phosphatidylcholine compared to the same genotype fed the control diet (Table 2).

Neutral lipids

The n–3 fatty acid supplementation significantly enhanced the quantities of n–3 fatty acids found in the neutral lipids of supraspinatus muscle (**Table 4.**) The very increased amounts of these highly unsaturated fatty acids in the MH homozygous and heterozygous animals were related to reduced levels of medium-chain saturated fatty acids in these animals. The comparatively higher incorporation of these fatty acids in the neutral lipids of MH homozygous and heterozygous animals also caused significant differences in the quantities of polyene fatty acids between animals fed the fish oil diet and animals fed the control diet. It is of interest to note that the relative amounts of lauric acid (C12:0) and myristic acid (C14:0) were elevated in all animals fed the control diet. Additionally, pentadecanoic acid (C15:0) increased in the MH heterozygous animals and palmitic acid (C16:0) increased in the MH homozygous animals fed the control diet (Table 4). With the exception of palmitic acid, these differences were caused by

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TABLE 2. Total lipid content, neutral lipid, total and individual phospholipid content in cardiac muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and swine fed a control diet

Content	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of wet muscle weight					
Total lipid	2.87 ± 0.09	2.78 ± 0.07	2.79 ± 0.07	2.73 ± 0.07	2.76 ± 0.08	2.79 ± 0.13
	μmol/g wet muscle weight					
Neutral lipids	2.90 ± 0.83	2.08 ± 0.63	1.91 ± 0.67	1.61 ± 0.67	2.36 ± 0.73	2.64 ± 1.23
Total phospholipids	11.95 ± 3.50	17.55 ± 2.68	15.10 ± 2.82	9.37 ± 2.82	11.88 ± 3.08	10.51 ± 5.22
Phosphatidylcholine	2.85 ± 0.56	3.68 ± 0.43	4.37 ± 0.45[a]	2.53 ± 0.45[b]	3.21 ± 0.49	2.43 ± 0.83
Phosphatidylethanolamine	1.81 ± 1.14	2.24 ± 0.87	2.50 ± 0.92	1.38 ± 0.92	1.11 ± 1.01	4.31 ± 1.70
Lyso-phosphatidylcholine	0.65 ± 1.00	1.25 ± 0.77	1.14 ± 0.81	0.55 ± 0.81	3.07 ± 0.88	0 ± 0
Lyso-phosphatidylethanolamine	2.93 ± 0.86	4.38 ± 0.66	3.24 ± 0.69	2.32 ± 0.69	2.00 ± 0.76	1.43 ± 1.28
Phosphatidylserine	0.57 ± 0.68	0.95 ± 0.52	0.62 ± 0.55	0.53 ± 0.55	0.23 ± 0.60	0.88 ± 1.02
Phosphatidylinositol	0.40 ± 0.61	1.61 ± 0.47	0.34 ± 0.49	0.43 ± 0.49	0.33 ± 0.54	0 ± 0
Cardiolipin	1.90 ± 0.75	2.52 ± 0.57	1.80 ± 0.60	1.09 ± 0.60	1.18 ± 0.66	1.20 ± 1.11
Sphingomyelin	0.84 ± 0.29	0.93 ± 0.22	1.08 ± 0.23	0.54 ± 0.23	0.76 ± 0.26	0.87 ± 0.43

Data are presented as least mean square values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between animals fed the high n–3 fatty acid diet and those fed the control diet.

the high relative amounts of these fatty acids in the control diet (Table 1).

In cardiac muscle only the MH heterozygous animals fed the high n–3 fatty acid diet showed significant higher levels of n–3 fatty acids compared to animals with the same genotype fed the control diet (7.32 ± 1.16% vs. 3.40 ± 1.16% of total fatty acids, least square mean values ± standard error, $P < 0.05$).

Phosphatidylcholine

A significant interaction between the amount of dietary n–3 fatty acids and the MH genotype was found for the relative amounts of n–3 fatty acids in the phosphatidylcholine of cardiac muscle. The dietary supplementation of n–3 fatty acids significantly enhanced the relative amounts of these fatty acids only in the MH heterozygous and the normal animals. In contrast, the MH homozygous animals fed the control diet showed an enhanced amount of n–3 fatty acids in the phosphatidylcholine of the heart muscle compared to the other genotypes and this amount was not further increased by the high n–3 fatty acid diet (**Table 5.**) In the MH heterozygous animals the increased incorporation of n–3 fatty acids was related to a decrease of linoleic acid (C18:2 n–6).

In the supraspinatus muscle, the n–3 fatty acid sup-

TABLE 3. Total lipid content, neutral lipid, total and individual phospholipid content in supraspinatus muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and swine fed a control diet

Content	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of wet muscle weight					
Total lipid	3.02 ± 0.51	2.54 ± 0.33	3.16 ± 0.34	3.13 ± 0.33	3.29 ± 0.37	4.07 ± 0.64
	μmol/g wet muscle weight					
Neutral lipids	22.97 ± 6.74	17.65 ± 4.38	26.37 ± 4.39	26.57 ± 4.38	23.40 ± 4.80	41.92 ± 8.63
Total phospholipids	3.67 ± 1.03	3.34 ± 0.67	3.67 ± 0.67	4.45 ± 0.67	5.48 ± 0.74	3.80 ± 1.32
Phosphatidylcholine	2.77 ± 0.67	2.31 ± 0.43	2.43 ± 0.44	3.20 ± 0.43	3.24 ± 0.48	2.78 ± 0.86
Phosphatidylethanolamine	0.62 ± 0.21	0.56 ± 0.14	0.63 ± 0.14	0.68 ± 0.14	0.96 ± 0.15	0.51 ± 0.27
Lyso-phosphatidylcholine	0.07 ± 0.05	0.08 ± 0.03	0.05 ± 0.03	0.06 ± 0.03	0.13 ± 0.04	0.16 ± 0.06
Lyso-phosphatidylethanolamine	0.01 ± 0.07	0.12 ± 0.04	0.15 ± 0.04	0.11 ± 0.04	0.14 ± 0.05	0.13 ± 0.08
Phosphatidylserine	0.08 ± 0.03	0.08 ± 0.02	0.14 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.03
Phosphatidylinositol	0.03 ± 0.03	0.05 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.13 ± 0.02	0.05 ± 0.04
Cardiolipin	0.08 ± 0.04	0.09 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.09 ± 0.05
Sphingomyelin	0.01 ± 0.22	0.05 ± 0.14	0.10 ± 0.14	0.07 ± 0.14	0.62 ± 0.16	0 ± 0

Data presented as least square mean values ± standard error.

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TABLE 4. Fatty acid profiles of neutral lipids in supraspinatus muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and those fed a control diet

Fatty Acids	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of total fatty acids					
C12:0	0.06 ± 0.13[a]	0.82 ± 0.09[b]	0.29 ± 0.09[a]	0.59 ± 0.08[b]	0.23 ± 0.09[a]	0.82 ± 0.17[b]
C14:0	1.62 ± 0.25[a]	2.83 ± 0.17[b]	1.80 ± 0.16[a]	2.62 ± 0.16[b]	1.92 ± 0.18[a]	3.14 ± 0.33[b]
C15:0	0.12 ± 0.03	0.06 ± 0.02	0.10 ± 0.02[a]	0.03 ± 0.02[b]	0.09 ± 0.02	0.07 ± 0.03
C16:0	23.91 ± 0.43[a]	25.42 ± 0.29[b]	24.18 ± 0.28	24.97 ± 0.28	24.89 ± 0.30	24.98 ± 0.57
C16:1	3.72 ± 0.24	3.21 ± 0.16	3.55 ± 0.16	3.47 ± 0.16	3.75 ± 0.17	3.56 ± 0.32
C17:0	0.42 ± 0.05	0.34 ± 0.03	0.41 ± 0.03	0.34 ± 0.03	0.39 ± 0.03	0.35 ± 0.06
C18:0	12.02 ± 0.80	12.84 ± 0.54	11.67 ± 0.52	12.08 ± 0.52	11.68 ± 0.57	12.19 ± 1.07
C18:1 *trans* 11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:1 *cis* 9	37.93 ± 1.48	38.82 ± 1.01	38.80 ± 0.96	40.90 ± 0.96	38.18 ± 1.06	39.95 ± 1.98
C18:1 *cis* 11	4.65 ± 0.29[a]	3.93 ± 0.20[b]	4.30 ± 0.19	4.22 ± 0.19	4.43 ± 0.21	3.92 ± 0.38
C18:2n–6	8.47 ± 0.94	7.98 ± 0.64	8.27 ± 0.61	7.00 ± 0.61	8.21 ± 0.67	7.19 ± 1.25
C18:3n–3	0.95 ± 0.09[a]	0.62 ± 0.06[b]	1.01 ± 0.06[a]	0.57 ± 0.06[b]	0.89 ± 0.06[a]	0.60 ± 0.12[b]
C18:4n–3	0.33 ± 0.05[a]	0.09 ± 0.03[b]	0.38 ± 0.03[a]	0.05 ± 0.03[b]	0.29 ± 0.03	0.18 ± 0.06
C20:0	0.20 ± 0.03	0.17 ± 0.02	0.15 ± 0.02[a]	0.22 ± 0.02[b]	0.20 ± 0.02	0.16 ± 0.04
C20:1	0.88 ± 0.03[a]	0.68 ± 0.02[b]	0.79 ± 0.02	0.82 ± 0.02	0.75 ± 0.02	0.78 ± 0.05
C20:4n–6	0.77 ± 0.10	0.76 ± 0.07	0.54 ± 0.07	0.67 ± 0.07	0.74 ± 0.07	0.67 ± 0.14
C20:3n–3	0.19 ± 0.03[a]	0.06 ± 0.02[b]	0.19 ± 0.02[a]	0.07 ± 0.02[b]	0.16 ± 0.02	0.12 ± 0.04
C20:5n–3	1.06 ± 0.08[a]	0.26 ± 0.06[b]	1.01 ± 0.05[a]	0.26 ± 0.05[b]	0.84 ± 0.06[a]	0.28 ± 0.11[b]
C22:0	n.d.	n.d.	n.d.	0.01 ± 0.01	0.02 ± 0.01	n.d.
C22:1	n.d.	n.d.	n.d.	0.01 ± 0.01	0.02 ± 0.01	n.d.
C22:4n–3	n.d.	0.04 ± 0.02	n.d.	0.03 ± 0.02	0.06 ± 0.02	0.02 ± 0.03
C24:0	0.90 ± 0.07[a]	0.41 ± 0.05[b]	0.88 ± 0.05[a]	0.42 ± 0.04[b]	0.77 ± 0.05[a]	0.38 ± 0.09[b]
C22:6n–3	1.67 ± 0.11[a]	0.50 ± 0.07[b]	1.50 ± 0.07[a]	0.48 ± 0.07[b]	1.34 ± 0.08[a]	0.50 ± 0.15[b]
Total n–3	4.19 ± 0.30[a]	1.53 ± 0.21[b]	4.09 ± 0.20[a]	1.43 ± 0.20[b]	3.51 ± 0.22[a]	1.68 ± 0.40[b]
Total n–6	9.24 ± 1.00	8.78 ± 0.68	8.81 ± 0.65	7.70 ± 0.65	9.01 ± 0.71	7.88 ± 1.34
Polyenic	13.44 ± 1.19[a]	10.30 ± 0.81[b]	12.91 ± 0.78[a]	9.13 ± 0.78[b]	12.52 ± 0.86	9.57 ± 1.60
Monoenic	47.19 ± 1.50	46.64 ± 1.02	47.44 ± 0.98	49.42 ± 0.98	47.12 ± 1.07	48.22 ± 2.01
Saturated	39.38 ± 0.91[a]	43.05 ± 0.62[b]	39.65 ± 0.59[a]	41.45 ± 0.59[b]	40.36 ± 0.65	42.22 ± 1.22

Data presented as least square mean values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between the animals fed the high n–3 fatty acid diet and those fed the control diet; n.d., not detectable.

plementation caused a significant increase in the amount of n–3 fatty acids found in the phosphatidylcholine of animals from all genotypes (**Table 6.**) This increased incorporation of n–3 fatty acids was related to a decrease of arachidonic acid (C20:4n–6) in the MH homozygous animals, a decrease of oleic acid (C18:1*cis*9) in MH homozygous and heterozygous animals and an increase of saturated fatty acids in the MH homozygous animals (Table 6). It is of interest to note that the amount of *cis*-vaccenic acid (C18:1*cis*11) was significantly elevated in all animals fed the fish oil diet.

Phosphatidylethanolamine

The highest levels of n–3 fatty acids in the phospholipids were found in the phosphatidylethanolamine fraction of the cardiac muscle. In the animals fed the fish oil diet, up to 26.7% of all fatty acids were n–3 fatty acids. The significantly increased levels of n–3 fatty acids were related to significantly decreased levels of arachidonic acid (C20:4n–6) in the MH homozygous and heterozygous animals (**Table 7**).

The n–3 fatty acid supplementation significantly enhanced the quantities of n–3 fatty acids found in the phosphatidylethanolamine of supraspinatus muscle in all genotypes. This was related to a significant decrease of linoleic acid (C18:2n–6) and arachidonic acid (C20:4n–6) in all animals. Higher levels of saturated fatty acids were noted in the phosphatidylethanolamine of the MH homozygous animals fed the fish oil diet (**Table 8**).

Lyso-phosphatidylcholine and lyso-phosphatidylethanolamine

In the cardiac lyso-phosphatidylcholine fraction, which also contained the plasmalogens, the relative amounts of n–3 fatty acids were significantly enhanced by the n–3 fatty acid supplementation in the MH heterozygous animals (**Table 9**). Comparable to the phosphatidylethanolamine, in all animals fed the n–3 fatty acid diet the relative amounts of n–3 fatty acids in the lyso-phosphatidylethanolamine fraction were significantly increased in the cardiac muscle. In supraspinatus muscle, significantly increased levels of n–3 fatty acids in lyso-phosphatidylethanolamine were only found in

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TABLE 5. Fatty acid profiles of phosphatidylcholine in cardiac muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and those fed a control diet

Fatty Acids	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of total fatty acids					
C12:0	0.29 ± 0.24	0.35 ± 0.18	0.37 ± 0.19	0.12 ± 0.19	0.30 ± 0.20	0.36 ± 0.34
C14:0	0.70 ± 0.26	0.72 ± 0.20	0.35 ± 0.21	0.36 ± 0.21	0.52 ± 0.23	0.47 ± 0.39
C15:0	0.03 ± 0.05	n.d.	n.d.	n.d.	n.d.	0.12 ± 0.08
C16:0	33.80 ± 1.44	31.79 ± 1.08	35.26 ± 1.13	34.16 ± 1.13	33.90 ± 1.24	34.27 ± 2.09
C16:1	0.87 ± 0.17	0.50 ± 0.14	0.75 ± 0.14^a	0.22 ± 0.14^b	1.08 ± 0.15	0.85 ± 0.25
C17:0	0.70 ± 0.26	0.49 ± 0.20	0.36 ± 0.21	0.13 ± 0.21	0.48 ± 0.23	1.11 ± 0.38
C18:0	10.59 ± 0.80	11.11 ± 0.60	8.99 ± 0.63	10.68 ± 0.63	10.23 ± 0.68	10.14 ± 1.16
C18:1 *trans* 11	0.07 ± 0.15	n.d.	n.d.	n.d.	n.d.	0.34 ± 0.22
C18:1 *cis* 9	17.20 ± 1.38	17.97 ± 1.03	18.01 ± 1.09	19.46 ± 1.09	20.72 ± 1.19	17.54 ± 2.01
C18:1 *cis* 11	4.98 ± 0.77	4.39 ± 0.57	5.95 ± 0.60^a	4.15 ± 0.60^b	5.74 ± 0.66	4.81 ± 1.11
C18:2n–6	22.54 ± 1.52	22.66 ± 1.14	20.00 ± 1.20^a	24.43 ± 1.20^b	17.18 ± 1.31	21.40 ± 2.21
C18:3n–3	0.06 ± 0.12	0.20 ± 0.09	0.25 ± 0.10	n.d.	0.18 ± 0.11	0.27 ± 0.18
C18:4n–3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:0	0.17 ± 0.30	0.14 ± 0.22	n.d.	n.d.	n.d.	0.58 ± 0.43
C20:1	0.08 ± 0.20	0.07 ± 0.15	n.d.	n.d.	n.d.	0.49 ± 0.29
C20:4n–6	1.47 ± 0.47^a	3.41 ± 0.35^b	2.16 ± 0.37	2.80 ± 0.37	2.47 ± 0.41	2.50 ± 0.69
C20:3n–3	n.d.	0.73 ± 0.22	0.03 ± 0.23	0.04 ± 0.23	0.18 ± 0.25	0.16 ± 0.42
C20:5n–3	3.67 ± 0.41	2.93 ± 0.31	4.36 ± 0.32^a	1.96 ± 0.32^b	4.50 ± 0.35^a	1.83 ± 0.60^b
C22:0	0.17 ± 0.34	n.d.	n.d.	n.d.	n.d.	0.77 ± 0.50
C22:1	0.10 ± 0.21	n.d.	n.d.	n.d.	n.d.	0.47 ± 0.30
C22:4n–3	n.d.	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.10
C24:0	2.06 ± 0.58	1.64 ± 0.43	1.48 ± 0.45	1.33 ± 0.46	1.24 ± 0.50	1.66 ± 0.84
C22:6n–3	0.78 ± 0.31	0.91 ± 0.23	1.70 ± 0.25^a	0.25 ± 0.25^b	1.55 ± 0.27^a	0.01 ± 0.45^b
Total n–3	4.18 ± 0.65	4.77 ± 0.48	6.35 ± 0.51^a	2.26 ± 0.51^b	6.41 ± 0.56^a	1.99 ± 0.94^b
Total n–6	24.01 ± 1.80	26.06 ± 1.35	22.16 ± 1.42^a	27.23 ± 1.42^b	19.65 ± 1.55	24.03 ± 2.62
Polyenic	28.19 ± 1.88	30.83 ± 1.41	28.51 ± 1.48	29.49 ± 1.48	26.06 ± 1.61	26.03 ± 2.73
Monoenic	23.31 ± 1.18	22.94 ± 0.89	24.69 ± 0.94	23.80 ± 0.94	27.41 ± 1.02	24.48 ± 1.72
Saturated	48.50 ± 1.08	46.23 ± 0.81	46.80 ± 0.85	46.71 ± 0.85	46.53 ± 0.93	49.49 ± 1.57

Data presented as least square mean values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between the animals fed the high n–3 fatty acid diet and those fed the control diet; n.d., not detectable.

the normal animals (Table 9). Furthermore, MH homozygous animals fed the fish oil diet showed significantly increased levels of saturated fatty acids in the lyso-phosphatidylethanolamine of the supraspinatus muscle **(Table 10).**

Phosphatidylserine and phosphatidylinositol

Dietary supplementation with n–3 fatty acids resulted in significant higher levels of n–3 fatty acids in the phosphatidylserine of the supraspinatus muscle from MH heterozygous animals (Table 9). In contrast to the MH heterozygous and to the normal animals, MH homozygous animals fed the fish oil diet showed significantly decreased amounts of n–6 fatty acids and increased amounts of saturated fatty acids in the phosphatidylinositol of supraspinatus muscle (Table 10 and **Table 11**).

Cardiolipin and sphingomyelin

The relative amounts of n–3 fatty acids in the sphingomyelin of the cardiac muscle were significantly enhanced by the dietary n–3 fatty acid supplementation in the MH heterozygous animals (Table 9). Interestingly, the MH homozygous animals fed the fish oil diet showed significantly lower amounts of n–3 fatty acids, but higher amounts of saturated fatty acids in the sphingomyelin of the supraspinatus muscle (Tables 9 and 10). In cardiolipin no supplementation effects could be detected in both tissues.

DISCUSSION

Swine susceptible to MH elicite a number of phenotypic properties that are considered desireable by the porcine industry (e.g., muscle hypertrophy, lower intermuscular fat content, and increased growth rates), but other properties are unwanted (e.g., poor meat quality) (25–27, 46). Although these features may be related to the underlying MH genotype, this has not been proved. Work is ongoing to determine how the MH genotype in swine relates to identified phenotypic changes and/or other innate differences. For example, the present study was designed to determine whether normal swine

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TABLE 6. Fatty acid profiles of phosphatidylcholine in supraspinatus muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and those fed a control diet

Fatty Acids	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of total fatty acids					
C12:0	n.d.	0.07 ± 0.11	0.26 ± 0.10	0.02 ± 0.10	0.22 ± 0.11	0.34 ± 0.21
C14:0	0.36 ± 0.15	0.64 ± 0.10	0.25 ± 0.10	0.45 ± 0.10	0.38 ± 0.11	0.46 ± 0.20
C15:0	0.24 ± 0.09	0.08 ± 0.06	0.20 ± 0.06	0.17 ± 0.06	0.16 ± 0.07	0.29 ± 0.13
C16:0	36.84 ± 0.95^a	33.88 ± 0.65^b	35.73 ± 0.62	34.51 ± 0.61	34.39 ± 0.68	34.63 ± 1.27
C16:1	1.11 ± 0.21	0.95 ± 0.14	1.21 ± 0.13	1.16 ± 0.13	1.33 ± 0.15	0.81 ± 0.28
C17:0	1.14 ± 0.25	0.58 ± 0.17	0.83 ± 0.16	1.12 ± 0.16	0.70 ± 0.18	0.83 ± 0.33
C18:0	9.39 ± 0.73	9.65 ± 0.50	8.40 ± 0.48	8.68 ± 0.47	8.37 ± 0.52	9.73 ± 0.98
C18:1 *trans* 11	0.12 ± 0.10	0.17 ± 0.07	n.d.	n.d.	0.03 ± 0.07	n.d.
C18:1 *cis* 9	11.46 ± 1.03^a	15.16 ± 0.71^b	11.56 ± 0.67^a	17.11 ± 0.67^b	11.02 ± 0.74	13.61 ± 1.39
C18:1 *cis* 11	5.63 ± 0.29^a	4.86 ± 0.20^b	5.75 ± 0.19^a	4.25 ± 0.19^b	6.42 ± 0.21^a	4.85 ± 0.39^b
C18:2n–6	25.16 ± 0.82	26.57 ± 0.56	25.76 ± 0.54	26.05 ± 0.54	26.07 ± 0.59	27.55 ± 1.11
C18:3n–3	0.97 ± 0.19	1.04 ± 0.13	1.13 ± 0.12	1.06 ± 0.12	1.37 ± 0.14	0.95 ± 0.25
C18:4n–3	0.15 ± 0.07	n.d.	0.06 ± 0.04	0.01 ± 0.04	0.13 ± 0.05	n.d.
C20:0	n.d.	n.d.	n.d.	n.d.	0.02 ± 0.01	n.d.
C20:1	0.15 ± 0.11	0.23 ± 0.07	0.34 ± 0.07^a	0.11 ± 0.07^b	0.30 ± 0.08	0.07 ± 0.15
C20:4n–6	1.67 ± 0.22^a	2.66 ± 0.15^b	1.79 ± 0.15	2.08 ± 0.14	1.96 ± 0.16	2.55 ± 0.30
C20:3n–3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:5n–3	3.58 ± 0.20^a	1.45 ± 0.14^b	3.95 ± 0.13^a	1.32 ± 0.13^b	3.52 ± 0.14^a	1.31 ± 0.27^b
C22:0	n.d.	0.01 ± 0.03	0.05 ± 0.03	n.d.	0.05 ± 0.04	n.d.
C22:1	n.d.	n.d.	n.d.	n.d.	0.02 ± 0.01	n.d.
C22:4n–3	0.02 ± 0.04	n.d.	n.d.	0.05 ± 0.03	0.04 ± 0.03	0.11 ± 0.06
C24:0	0.71 ± 0.20	0.83 ± 0.14	0.92 ± 0.13	0.93 ± 0.13	1.07 ± 0.14	1.09 ± 0.27
C22:6n–3	1.34 ± 0.33	1.19 ± 0.23	1.80 ± 0.22^a	0.93 ± 0.22^b	2.42 ± 0.24^a	0.95 ± 0.45^b
Total n–3	6.04 ± 0.37^a	3.67 ± 0.26^b	6.94 ± 0.24^a	3.32 ± 0.24^b	7.44 ± 0.27^a	3.21 ± 0.50^b
Total n–6	26.85 ± 0.91^a	29.23 ± 0.62^b	27.55 ± 0.59	28.17 ± 0.59	28.08 ± 0.65	30.20 ± 1.21
Polyenic	32.89 ± 1.11	32.90 ± 0.76	34.49 ± 0.72^a	31.49 ± 0.72^b	35.52 ± 0.79	33.42 ± 1.49
Monoenic	18.47 ± 1.13^a	21.37 ± 0.77^b	18.87 ± 0.74^a	22.63 ± 0.73^b	19.12 ± 0.81	19.27 ± 1.51
Saturated	48.64 ± 0.88^a	45.73 ± 0.60^b	46.65 ± 0.57	45.88 ± 0.57	45.36 ± 0.63	47.31 ± 1.18

Data presented as least square mean values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between the animals fed the high n–3 fatty acid diet and those fed the control diet; n.d., not detectable.

and those susceptible to MH (both heterozygous and homozygous animals) differ in their abilities to incorporate n–3 fatty acids into their skeletal and heart muscles. In this study, we observed that n–3 fatty acids were largely incorporated into neutral lipids and phospholipids of heart and skeletal muscle of swine whose diet had been supplemented with 5% fish oil. The control groups for each genotype received a diet that was considered to have the same nutritional value. In general, total lipid content, neutral lipid content, total phospholipid content, and individual phospholipid content were not affected by the supplementation. Only MH heterozygous animals fed the fish oil diet showed higher amounts of phosphatidylcholine in cardiac muscle, compared to the control animals.

The incorporation of n–3 fatty acids within neutral lipids was greatest in the supraspinatus muscle. The enhanced levels of n–3 fatty acids were related to decreased levels of medium chain saturated fatty acids. Therefore, the fatty acid pattern of neutral lipids in supraspinatus muscle showed a direct relation to the fatty acid pattern of the diets, where the fish oil diet was low and the control diet was high in medium chain saturated fatty acids. This was probably due to the direct incorporation of the dietary fatty acids into the triglycerides of the intramuscular adipocytes.

In general, the increased incorporation of n–3 fatty acids into the different phospholipids was related to a decreased content of linoleic and/or arachidonic acid. In the phosphatidylcholine of supraspinatus muscle, levels of oleic acid were also reduced. These results are consistent with the reports of other authors who have indicated a displacement especially of arachidonic acid by n–3 fatty acids in different phospholipids, in both in vivo and in vitro studies (47–51). The displacement of n–6 fatty acids by n–3 fatty acids was most pronounced in phosphatidylcholine, phosphatidylethanolamine, and lyso-phosphatidylethanolamine. Little or no displacement could be noted in lyso-phosphatidylcholine, phosphatidylinositol, phosphatidylserine, cardiolipin, and sphingomyelin. Similar results for pooled phospholipids or several phospholipid species are reported by other authors (50, 52.)

Although, tachycardia is considered as a reliable and

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TABLE 7. Fatty acid profiles of phosphatidylethanolamine in cardiac muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and those fed a control diet

Fatty Acids	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
	% of total fatty acids					
C12:0	0.07 ± 0.40	0.54 ± 0.30	0.54 ± 0.31	0.31 ± 0.31	n.d.	n.d.
C14:0	0.21 ± 0.48	0.92 ± 0.36	0.34 ± 0.38	0.26 ± 0.38	0.06 ± 0.41	0.94 ± 0.69
C15:0	n.d.	n.d.	n.d.	n.d.	n.d.	0.01 ± 0.01
C16:0	9.61 ± 1.97	9.84 ± 1.47	11.47 ± 1.55	8.98 ± 1.55	7.56 ± 1.69	14.27 ± 2.86
C16:1	0.23 ± 0.30	0.75 ± 0.22	0.36 ± 0.24	0.13 ± 0.24	n.d.	0.89 ± 0.44
C17:0	n.d.	0.84 ± 0.21	0.29 ± 0.22	0.22 ± 0.22	0.21 ± 0.24	0.10 ± 0.41
C18:0	28.44 ± 1.89	25.88 ± 1.42	27.66 ± 1.49	29.04 ± 1.50	31.26 ± 1.63	26.86 ± 2.75
C18:1 *trans* 11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:1 *cis* 9	9.23 ± 2.90	10.67 ± 2.17	10.96 ± 2.29	8.49 ± 2.29	5.39 ± 2.50[a]	15.75 ± 4.23[b]
C18:1 *cis* 11	2.03 ± 0.45	2.20 ± 0.33	2.25 ± 0.35	1.48 ± 0.35	1.72 ± 0.38	1.21 ± 0.65
C18:2n–6	15.12 ± 1.35	16.49 ± 1.01	14.41 ± 1.07	17.06 ± 1.07	14.48 ± 1.16	9.80 ± 1.97
C18:3n–3	0.03 ± 0.10	0.11 ± 0.08	0.06 ± 0.08	n.d.	n.d.	0.28 ± 0.15
C18:4n–3	0.01 ± 0.03	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.04
C20:0	0.01 ± 0.02	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.02
C20:1	0.06 ± 0.14	0.20 ± 0.10	0.08 ± 0.11	n.d.	n.d.	0.19 ± 0.20
C20:4n–6	7.67 ± 1.98[a]	12.98 ± 1.48[b]	7.41 ± 1.56[a]	14.72 ± 1.56[b]	10.23 ± 1.70	12.88 ± 2.88
C20:3n–3	0.32 ± 0.47	0.72 ± 0.35	0.02 ± 0.37	0.53 ± 0.37	n.d.	1.30 ± 0.68
C20:5n–3	13.56 ± 1.35[a]	8.20 ± 1.01[b]	12.43 ± 1.06	9.45 ± 1.07	16.30 ± 1.16[a]	7.84 ± 1.96[b]
C22:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:4n–3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C24:0	4.52 ± 0.84	3.63 ± 0.63	2.86 ± 0.66	3.63 ± 0.66	2.55 ± 0.72	2.80 ± 1.22
C22:6n–3	8.89 ± 0.98[a]	6.01 ± 0.74[b]	8.87 ± 0.78[a]	5.72 ± 0.78[b]	10.54 ± 0.84[a]	4.78 ± 1.43[b]
Total n–3	22.81 ± 2.14[a]	15.05 ± 1.60[b]	21.37 ± 1.69[a]	15.70 ± 1.69[b]	26.72 ± 1.84[a]	14.25 ± 3.11[b]
Total n–6	22.80 ± 2.74	29.47 ± 2.05	21.82 ± 2.17[a]	31.77 ± 2.17[b]	24.71 ± 2.36	22.68 ± 3.99
Polyenic	45.61 ± 4.05	44.52 ± 3.03	43.19 ± 3.20	47.47 ± 3.20	51.43 ± 3.48[a]	36.93 ± 5.90[b]
Monoenic	11.55 ± 3.48	13.83 ± 2.60	13.65 ± 2.74	10.10 ± 2.74	6.95 ± 2.99	18.05 ± 5.06
Saturated	42.85 ± 1.27	41.65 ± 0.95	43.16 ± 1.01	42.43 ± 1.01	41.62 ± 1.09	45.02 ± 1.85

Data presented as least square mean values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between the animals fed the high n–3 fatty acid diet and those fed the control diet; n.d., not detectable.

early sign of an MH episode, most previous reports also suggest that the cardiovascular changes observed during acute MH are secondary to the abnormal response of skeletal muscles. This is consistent with recent data that suggest it is unlikely that cardiac tissue expresses the same mutation in the calcium-release channel found in skeletal muscle of susceptible animals (53). The ryanodine-sensitive calcium release channel in heart is encoded on a chromosome that is distant from the one for the skeletal muscle release channel. Nevertheless, tachycardia and succinylcholine-induced hypotension are considered as early signs of an onset of an MH episode in swine (54). On the other hand, a recent report showed that only isoproterenol had a differential inotropic effect on trabeculae isolated from pigs susceptible to MH compared to those from normal animals. Caffeine, halothane, succinylcholine, and phenylephrine had similar effects in both animal groups (55). These authors concluded that the cardiac manifestations during malignant hyperthermic crises result from a primary myocardial abnormality. Interestingly, the phospholipid and fatty acid composition differed significantly between normal swine and those susceptible to MH not only in skeletal muscle but also in cardiac muscle (28, 56). However, the results from previous studies have not been consistent. Wahle et al. (56) found 70% less polyunsaturated fatty acids in the total phospholipids of cardiac left ventricle from MH susceptible pigs compared to normal animals. These authors concluded that there was an enhanced peroxidation of n–6 and n–3 fatty acids in these animals. In our study we found a significant difference between the animals for the incorporation of eicosapentaenoic acid and docosahexaenoic acid in the phosphatidylcholine of cardiac muscle. In contrast to the MH heterozygous and to the normal animals, the MH homozygous animals showed no effect of dietary supplementation on the levels of n–3 fatty acids. However, amounts of these fatty acids were higher compared to the amounts found in the other genotypes fed the control diet. Although such differences in membrane composition may be secondary, they nonetheless may play a role in the presentation and/or proliferation of a malignant hyperthermic episode.

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TABLE 8. Fatty acid profiles of phosphatidylethanolamine in supraspinatus muscle of swine with different MH genotypes fed a high n–3 fatty acid diet and those fed a control diet

Fatty Acids	MH Homozygous		MH Heterozygous		Normal Animals	
	Fish Oil n = 5	Control n = 8	Fish Oil n = 7	Control n = 7	Fish Oil n = 6	Control n = 6
			% of total fatty acids			
C12:0	n.d.	0.23 ± 0.41	0.58 ± 0.39	0.21 ± 0.39	0.19 ± 0.43	1.29 ± 0.81
C14:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C15:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16:0	4.72 ± 0.87	3.73 ± 0.59	4.88 ± 0.57	4.00 ± 0.57	3.31 ± 0.62	3.88 ± 1.17
C16:1	0.01 ± 0.05	0.02 ± 0.03	0.04 ± 0.03	n.d.	0.05 ± 0.03	0.01 ± 0.06
C17:0	0.38 ± 0.53	1.25 ± 0.36	1.07 ± 0.35	0.36 ± 0.35	0.84 ± 0.38	0.62 ± 0.71
C18:0	42.27 ± 1.67	38.99 ± 1.14	38.83 ± 1.09	38.05 ± 1.09	37.03 ± 1.20	37.64 ± 2.24
C18:1 *trans* 11	n.d.	0.01 ± 0.03	n.d.	n.d.	0.06 ± 0.03	n.d.
C18:1 *cis* 9	6.31 ± 1.22	7.31 ± 0.83	7.04 ± 0.80	8.31 ± 0.80	5.10 ± 0.88	7.13 ± 1.64
C18:1 *cis* 11	0.67 ± 1.07	0.77 ± 0.73	1.40 ± 0.70	1.08 ± 0.69	1.64 ± 0.76	2.91 ± 1.43
C18:2n–6	20.76 ± 1.03[a]	23.68 ± 0.70[b]	21.44 ± 0.67[a]	24.60 ± 0.67[b]	20.23 ± 0.74[a]	24.08 ± 1.38[b]
C18:3n–3	0.06 ± 0.19	0.17 ± 0.13	0.17 ± 0.13	0.22 ± 0.12	0.38 ± 0.14	0.19 ± 0.26
C18:4n–3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:0	0.01 ± 0.38	0.06 ± 0.26	n.d.	n.d.	0.62 ± 0.27	n.d.
C20:1	n.d.	0.58 ± 0.27	n.d.	0.01 ± 0.26	0.44 ± 0.28	n.d.
C20:4n–6	6.38 ± 0.92[a]	11.67 ± 0.63[b]	5.89 ± 0.60[a]	10.26 ± 0.60[b]	7.26 ± 0.66[a]	11.97 ± 1.24[b]
C20:3n–3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:5n–3	9.83 ± 0.93[a]	4.74 ± 0.63[b]	10.00 ± 0.61[a]	5.06 ± 0.60[b]	10.21 ± 0.67[a]	5.37 ± 1.25[b]
C22:0	0.02 ± 0.42	0.08 ± 0.29	n.d.	n.d.	0.69 ± 0.30	n.d.
C22:1	0.01 ± 0.36	0.06 ± 0.25	n.d.	n.d.	0.60 ± 0.26	n.d.
C22:4n–3	n.d.	0.02 ± 0.06	n.d.	n.d.	0.22 ± 0.06	0.09 ± 0.12
C24:0	2.21 ± 0.58	2.58 ± 0.39	2.05 ± 0.38[a]	3.43 ± 0.37[b]	3.26 ± 0.41	3.44 ± 0.77
C22:6n–3	6.37 ± 0.91[a]	4.06 ± 0.62[b]	6.65 ± 0.59[a]	4.44 ± 0.59[b]	7.87 ± 0.65[a]	4.12 ± 1.22[b]
Total n–3	16.26 ± 1.01[a]	8.97 ± 0.69[b]	16.82 ± 0.66[a]	9.71 ± 0.66[b]	18.47 ± 0.73[a]	9.68 ± 1.36[b]
Total n–6	27.13 ± 1.20[a]	35.37 ± 0.82[b]	27.33 ± 0.78[a]	34.86 ± 0.78[b]	27.70 ± 0.86[a]	36.14 ± 1.61[b]
Polyenic	43.39 ± 1.66	44.34 ± 1.14	44.15 ± 1.09	44.57 ± 1.08	46.16 ± 1.19	45.82 ± 2.23
Monoenic	7.01 ± 1.55	8.75 ± 1.06	8.46 ± 1.01	9.39 ± 1.01	7.89 ± 1.11	8.90 ± 2.08
Saturated	49.60 ± 1.04[a]	46.91 ± 0.71[b]	47.39 ± 0.68	46.03 ± 0.68	45.94 ± 0.75	45.28 ± 1.40

Data presented as least square mean values ± standard error. Within each genotype different superscript letters indicate a significant difference ($P < 0.05$) between the animals fed the high n–3 fatty acid diet and those fed the control diet; n.d., not detectable.

It is interesting to note that it was previously shown that enrichment of cellular phospholipids with docosahexaenoic acid was found to inhibit the calcium response by acting as a modulator of the L-type calcium channel (39). In addition, it has been shown that both eicosapentaenoic acid and docosahexaenoic acid modulate post-receptor signaling pathways and formation of second messengers, involved in the mobilization of intracellular calcium such as inositol trisphosphate (40). Further, increased resting inositol trisphosphate levels in MH porcine skeletal muscles were reported by several authors (57–59). These authors concluded that a significant fraction of the increases in cytoplasmic calcium came from nonmitochondrial intracellular stores,

TABLE 9. Differences in n–3 fatty acid content in cardiac and supraspinatus muscle samples from animals with different MH genotypes

	Cardiac Muscle			Supraspinatus Muscle		
	MH Homozyg.	MH Heterozyg.	Normal Animals	MH Homozyg.	MH Heterozyg.	Normal Animals
Phosphatidylcholine	−0.59	+4.09[a]	+4.42[a]	+2.37[a]	+3.62[a]	+4.23[a]
Phosphatidylethanolamine	+7.76[a]	+5.67[a]	+12.47[a]	+7.29[a]	+7.11[a]	+8.79[a]
Lyso-phosphatidylcholine	+4.23	+7.38[a]	+3.87	−5.49	+0.75	+26.89
Lyso-phosphatidylethanolamine	+9.75[a]	+11.82[a]	+9.72[a]	−9.66	+6.94	+42.96[a]
Phosphatidylserine	+0.30	−1.88	+0.52	+3.98	+11.16[a]	+15.23
Phosphatidylinositol	−1.99	+2.93	+2.12	−0.87	+1.20	+20.38
Cardiolipin	−1.95	−0.68	−1.56	+0.02	+0.69	+7.99
Sphingomyelin	+2.41	+6.16[a]	+5.50	−15.78[a]	+3.25	+8.47
Neutral lipids	+2.47	+3.92[a]	+2.87	+2.66[a]	+2.66[a]	+1.83[a]

Differences are calculated as % n–3 fatty acids in the fish oil group minus % n–3 fatty acids in the control group.
[a]Significant difference ($P < 0.05$) in relative n–3 fatty acid content between animals fed the fish oil diet and those fed the control diet.

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TABLE 10. Differences in saturated fatty acid content in cardiac and supraspinatus muscle samples from animals with different MH genotypes

	Cardiac Muscle			Supraspinatus Muscle		
	MH Homozyg.	MH Heterozyg.	Normal Animals	MH Homozyg.	MH Heterozyg.	Normal Animals
Phosphatidylcholine	+2.27	+0.09	−2.96	+2.91*	+0.77	−1.95
Phosphatidylethanolamine	+1.20	+0.73	−3.40	+2.69*	+1.36	+0.66
Lyso-phosphatidylcholine	−3.47	−4.72	+8.23	+33.48	+14.95	−17.45
Lyso-phosphatidylethanolamine	+0.91	+1.21	+0.19	+44.42*	+2.28	−42.14
Phosphatidylserine	−0.83	−1.27	−9.86	−0.83	−4.35	+0.16
Phosphatidylinositol	+7.92	−3.21	−11.00	+54.10*	−8.37	−8.86
Cardiolipin	+1.88	−0.18	+8.44	−6.97	+6.19	−1.43
Sphingomyelin	+8.27	−6.11	−2.48	+44.85*	+7.45	−36.59
Neutral lipids	+1.48	−2.60	−0.62	−3.67*	−1.80*	−1.86

Differences are calculated as % saturated fatty acids in the fish oil group minus % saturated fatty acids in the control group.
*Significant difference ($P < 0.05$) in relative saturated fatty acid content between animals fed the fish oil diet and those fed the control diet.

induced by increased levels of inositol trisphosphate. The generation of inositol phosphates, however, may be reduced by n–3 fatty acids (39). While arachidonic acid and eicosapentaenoic acid stimulate the activity of protein kinase C, it is inhibited by docosahexaenoic acid. A shift from n–6 to n–3 fatty acids, especially to docosahexaenoic acid may therefore reduce protein kinase C-induced cell responses (e.g., cell proliferation) (39). Furthermore, ATPase activity in cardiac sarcoplasmic reticulum from mice revealed that this enzyme was less active in animals fed a fish oil diet as compared to animals with a control diet rich in n–6 fatty acids (40). The potential inhibition of protein kinase C and ATPase activities by n–3 fatty acids compared to arachidonic acid, as well as the reduction of receptor-mediated rises in intracellular calcium and generation of inositol phosphates, indicate that n–3 fatty acids may counteract intracellular processes associated with the MH gene.

Compared to the dietary-induced variation of the n–3 and n–6 fatty acid composition, the influence of the different MH genotype of the animals on the relative amounts of saturated fatty acids in the membrane lipids of supraspinatus muscle was very specific. Whereas in the neutral lipids the relative amounts of these fatty acids were significantly lower in MH homozygous and heterozygous animals fed the fish oil diet, only MH homozygous animals fed the fish oil diet showed significantly higher levels of saturated fatty acids in the phosphatidylcholine, phosphatidylethanolamine, lyso-phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin. Whereas arachidonic acid was found to increase the calcium release from the sarcoplasmic reticulum of MH skeletal muscle, short chain derivatives and saturated fatty acids were without effect (60). Therefore, the altered incorporation of saturated and n–3 fatty acids may be a repair mechanism to maintain cellular functions and structures in MH animals.

In conclusion, the difference between normal swine and swine with the genetic mutation for MH to incorporate n–3 fatty acids in either cardiac and skeletal muscles may imply significant modifications in overall lipid metabolism. Such differences may underlie the phenotypic characteristics of enhanced muscle mass and re-

TABLE 11. Differences in n–6 fatty acid content in cardiac and supraspinatus muscle samples from animals with different MH genotypes

	Cardiac Muscle			Supraspinatus Muscle		
	MH Homozyg.	MH Heterozyg.	Normal Animals	MH Homozyg.	MH Heterozyg.	Normal Animals
Phosphatidylcholine	−2.05	−5.07*	−4.38	−2.38*	−0.62	−2.12
Phosphatidylethanolamine	−6.67	−9.95*	+2.03	−8.24*	−7.53*	−8.44*
Lyso-phosphatidylcholine	−1.75	−2.56	−16.60	+11.79	−0.14	−8.44
Lyso-phosphatidylethanolamine	−5.66	−12.34*	−6.09	−17.25*	−13.86	+5.26
Phosphatidylserine	−4.91	+3.37	+13.68	+0.09	−4.29	−5.23
Phosphatidylinositol	−4.91	+7.76	+3.02	−25.19*	−6.28	−6.17
Cardiolipin	−1.66	−0.67	+0.84	+3.04	−7.12	+1.55
Sphingomyelin	−9.96*	−6.41	−1.55	−4.89	+1.97	+4.08
Neutral lipids	−5.76	+2.49	+1.25	+0.46	+1.11	+1.13

Differences are calculated as % n–6 fatty acids in the fish oil group minus % n–6 fatty acids in the control group.
*Significant difference ($P < 0.05$) in relative n–6 fatty acid content between animals fed the fish oil diet and those fed the control diet.

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duced intermuscular fat content in swine susceptible to MH and be related to an enhanced response of the cardiovascular system to a MH event.

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