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Artificial Rearing of Infant Mice Leads to n-3 Fatty Acid Deficiency in Cardiac, Neural and Peripheral Tissues

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Abstract

The ability to control the fatty acid content of the diet during early development is a crucial requirement for a one-generation model of docosahexaenoic acid (DHA; 22:6n3) deficiency. A hand feeding method using artificial rearing (AR) together with sterile, artificial milk was employed for feeding mice from postnatal day 2–15. The pups were fed an n-3 fatty acid adequate (3% α -linolenic acid (LNA; 18:3n3) + 1% 22:6n3) or a deficient diet (0.06% 18:3n3) with linoleic acid (LA; 18:2n6) as the only dietary source of essential fatty acids by AR along with a dam-reared control group (3.1% 18:3n3). The results indicate that restriction of n-3 fatty acid intake during postnatal development leads to markedly lower levels of brain, retinal, liver, plasma and heart 22:6n3 at 20 weeks of age with replacement by docosapentaenoic acid (DPAn6; 22:5n6), arachidonic acid (ARA; 20:4n6) and docosatetraenoic acid (DTA; 22:4n6). A detailed analysis of phospholipid classes of heart tissue indicated that phosphatidylethanolamine, phosphatidylcholine and cardiolipin were the major repositories of 22:6n3, reaching 40, 29 and 15%, respectively. A novel heart cardiolipin species

containing four 22:6n3 moieties is described. This is the first report of the application of artificially rearing to mouse pup nutrition; this technique will facilitate dietary studies of knockout animals as well as the study of essential fatty acid (EFA) functions in the cardiovascular, neural and other organ systems.

Keywords

Artificial rearing; Artificial mouse milk; Docosahexaenoic acid; Docosapentaenoic acid; Brain; Heart; Retina; Liver; Plasma; Phospholipid class

Introduction

Nutritional studies during the early postnatal period are difficult to conduct since mammals are dependent upon maternal breast milk for nourishment and the breast milk composition cannot be controlled since it differs from the maternal diet. The artificial rearing (AR) method permits precise control of dietary essential fatty acids when used in combination with artificial milk and allows for a rapid induction of neural 22:6n3 deficiency in the newborn rat [1–3]. Hoshiba has introduced a new type of nursing bottle for use in AR employing hand feeding that supports excellent pup survival [4] and recently it has been adapted for essential fatty acid research [3,5]. This work has employed rats; however, mice are the most widely used animal in molecular biological work where genetic mutants are readily available. Thus, a practical method of artificially rearing mouse pups is desirable for neonatal nutrition.

Experimental animal, human and cell culture studies have reported anti-arrhythmic actions of omega 3 fatty acids [6]. Ku et al. [7] showed that the recovery of cardiac function after cold storage was impaired in hyperlipidemic rats fed a high-fat diet, but was restored in the presence of omega-3 fatty acids. The administration of a lipid preparation (medium chain-triacylglycerols (MCT): fish oil (FO) or MCT: triolein (OO)) improved cardiac function during aerobic reperfusion post-ischemia. In addition to the previous effect; the bolus injection of MCT:FO opposed the deleterious effect of long-term n-3 fatty acid deficiency and, in this respect, was superior to MCT:OO [8]. In a recent study by Portois et al. [9] the alteration of cardiac function in n-3 deficient rats and its improvement after injection of MCT:FO emulsion coincides with parallel changes in heart lipid fatty acid content and pattern.

In humans, Mori et al. [10] have also shown that compared with a placebo, heart rate was significantly reduced by 22:6n3 and increased by eicosapentaenoic acid (EPA; 20:5n3). The beat-to-beat variability correlated directly with the 22:6n3 content of platelet membranes in survivors of myocardial infarction [11]. The recent GISSI-Prevention study of 11,324 patients showed a marked decrease in the risk of sudden cardiac death as well as a reduction in all-cause mortality in the group taking omega-3 fatty acid ethyl esters, despite the use of other secondary prevention drugs, including beta-blockers and lipid-lowering therapy [12].

The fatty acid composition of myocardial membrane phospholipids is sensitive to the type of fatty acids consumed in the diet. Indeed, the myocardium and the myocardial membrane phospholipids are rich in n-3 PUFA after feeding fish oils [13–15]. Kramer [16] observed no effect of dietary lipids on the proportions of the major phospholipids of rat heart after feeding various plant oil supplements. These observations thus indicate that dietary lipid induced changes in the fatty acid composition of cardiac phospholipids are due primarily to changes in the fatty acid content of the lipids, rather than to altering lipid class profiles. Garg et al. [17] demonstrated for the first time that short-term supplementation with fish oil concentrate results in significant incorporation of long chain n-3 PUFA with a concomitant depletion of the eicosanoid substrate 20:4n6 in the human atrium.

In the present study, an AR system was employed to produce mice with lower levels of brain 22:6n3 in the first generation by feeding diets with only 18:2n6 as a source of essential fatty acids; a second group of mice were fed an identical diet to which 18:3n3 and 22:6n3 were substituted for a portion of the oleate (18:1n9). The fatty acid content of neural and peripheral tissues is presented.

Materials and Methods

Animals and Study Design

Time-pregnant, female ICR mice of 2-day gestational age were obtained from Harlan (Indianapolis, IN) and immediately placed on a diet (maternal diet) containing 3.1% 18:3n3 as the source of n-3 fatty acids (Table 1). They were maintained in our animal facility under conventional conditions with controlled temperature (23 ± 1 °C) and illumination (12 h; 06.00–18.00 h) and water was provided ad libitum. At postnatal day (PND) 2, one male pup was selected from various litters, born within a 48 h time window and randomly allocated into one of two experimental groups with the constraint that the groups had an equal mean body weight. A third male group served as another reference group and was allowed to suckle from dams fed on the maternal diet (dam-reared group; $n = 16$) containing 3.1 wt % 18:3n3. The two experimental groups were artificially fed one of the two artificial milks: n-3 fatty acid deficient (n-3 Def; $n = 14$) milk (18:2n6 only added as EFA) or an identical milk except that 3.1 wt % 18:3n3 and 1 wt % 22:6n3 were added (n-3 Adq; $n = 14$) in place of some of the monounsaturated fat (Table 2). All experimental procedures were approved by the Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism, National Institute of Health.

Artificial Rearing System

The AR procedure used was a hand-feeding technique with a newly developed nursing bottle as described by Hoshiba [4] and recently adapted for essential fatty acid research [3,5]. The AR system consisted of a custom made nursing bottle, container, cage, digital microprocessor incubator (Quincy Lab, Inc., Chicago, IL) with a thermo-hygrometer (Sper Scientific Ltd., Scottsdale, AZ).

Artificial Milk

The artificial milk formula was modified from the method of Yajima et al. [18,19]. Table 2 shows the ingredients used as well as their commercial sources. Casein, whey protein hydrolysate and whey protein were used as protein sources and lactose was used as the carbohydrate. The protein sources were carefully chosen so as not to introduce significant levels of n-3 fatty acids, as this is often the major source of introduction of these components into n-3 fatty acid deficient diets [25]. For complete dissolution, all ingredients were mixed in the order described by Kanno et al. [20] so as to minimize precipitation. The milk was then homogenized two times under high pressure (120 kg/cm^2) using a homogenizer with a two-stage valve (Model #HP50–250 FES International, Pomona, CA) that had been cleaned by rinsing with 0.1 M NaOH and then neutralized with sterilized water. The homogenized milk was tyndallized twice at 63 °C for 30 min, 6 h apart. The complete milk was poured into 50 mL sterilized polypropylene bottles (Falcon, San Jose, CA) and stored at -40 °C. The milks contained 16 wt % lipids composed of saturated fat (medium chain triglycerides and hydrogenated coconut oil) and the ethyl ester form of purified unsaturated fatty acids (Nu-Chek Prep, Inc., Elysian, MN). The n-3 fatty acid deficient milk (n-3 Def) contained 16.7% of 18:2n6 and 0.06% of 18:3n3 and the n-3 fatty acid adequate (n-3 Adq) milk contained 16.9% of 18:2n6 and 3.1% 18:3n3 and 0.93% of 22:6n3 upon actual analysis (Table 2). The addition of the 18:3n3 and 22:6n3 to the n-3 Adq diet was substituted for an equal quantity of ethyl 18:1n9; this introduced only a 4% difference in 18:1n9 content between the two diets. Saturated,

monounsaturated, and 18:2n6 were balanced leaving only the level of 18:3n3 and 22:6n3 as a dietary variable. Insulin-Like Growth Factor-I (IGF-I), (Fitzgerald Industries International, Concord, MA) was added to the milk as studies suggest that milk-borne IGF-I is important in modulation of somatic and gastrointestinal tract growth in the neonatal rat [21]. The IGF-I was added to the artificial mouse milk in the following manner: the IGF-I (10 µg in 0.5 mL of 0.1 M acetic acid) was added to Pyrex screw cap culture tubes (13 × 100 mm, Corning). Tube contents were lyophilized and stored at −20 °C until use. Each morning, 0.2 mL of Tris buffer (50 mM, pH 7.4) was added to each tube and subjected to gentle mixing, then transferred into sterile polypropylene bottles with 20 mL of artificial milk to achieve a final IGF-I concentration of 500 ng IGF-I/mL of artificial milk.

Pelleted Diets—The three pelleted diets used were based on the AIN-93 [22] formulation with several modifications to obtain the extremely low n-3 fatty acid level required in this study (Table 1). The diets were obtained commercially and used a cold pelleting process to preserve unsaturated fats (Dyets, Bethlehem, PA). Pups were weaned to pelleted diets with a fat composition similar to that fed during the AR period; the dam-reared group was weaned onto the same diet as their dams and they were maintained on these diets until they were killed. All three diets contained 10 wt % fat and had a similar content of 18:2n6. The fatty acid data in Tables 1 and 2 represent actual gas chromatographic (GC) analyses of the entire diet (rather than theoretical values or analysis of just the lipid mixtures).

Lipid Extraction

The mice were killed by decapitation at 20 weeks of age, tissue samples including brain, liver, heart, plasma and retina were collected and stored at −80 °C. The total lipid extracts of tissues were prepared according to the method of Folch et al. [23]. Aliquots of the lipid extracts were transmethyalted with 14% BF₃-methanol (Alltech Associates, Deerfield, IL) at 100 °C for 60 min by a modification of the method of Morrison and Smith [24] with hexane addition [25]. The total lipids in the artificial milk and pelleted diets were transmethyalted by the method of Lepage and Roy [26] as this is more accurate when shorter chain fatty acids (e.g. 8–12C) occur at appreciable levels. The fatty acid methyl esters were analyzed by a fast gas chromatographic method as previously described [27]. Data were expressed as a percentage of total fatty acid weight (% by wt) or referenced to an internal standard and expressed as an absolute concentration (µg/mg tissue wet weight).

High Performance Liquid Chromatography (HPLC) Separations of Heart Lipid Classes

HPLC Procedures—Normal-phase HPLC was performed using a HP1100 consisting of a quaternary pump, online degasser, autosampler and variable wavelength detector (Agilent Technologies, Wilmington, DE). The column was an Advantage silica 5 µm (60 Å) 250 × 4.6 mm purchased from Thomson Instrument Company (Clear Brook, VA). A guard column of 4.0 × 10 mm packed with silica was used in conjunction with the analytical column.

An aliquot of 0.3–0.6 mg of the heart tissue lipid extract was dissolved in 20 µL chloroform and injected into the HPLC system. Phospholipid separations were achieved with a solvent system consisting of hexane:2-propanol:25 mM potassium acetate (pH 7.0):ethanol:glacial acetic acid (250:607:62: 100:0.6) (by volume) (Buffer A) and hexane:2-propanol:25 mM potassium acetate (pH 7.0):acetonitrile:glacial acetic acid (442:490:62:25:0.6) (by volume) (Buffer B) at 50 °C using a modification of the method of Lesnefsky et al. [28]. The first 65 min of the elution scheme was designed to separate various lipid classes, starting with Buffer A for 15 min for elution of neutral lipids (which included triacylglycerol, cholesterol, and cholesterol esters) and subsequently phosphatidylethanolamine followed by a linear gradient from A to B for elution of phosphatidylinositol and cardiolipin. The flow rate was increased from 1.0 to 1.25 mL/min at 26 min and then held constant until the end of the elution scheme

for elution of phosphatidylserine and phosphatidylcholine. A 30 min period with Buffer A at the end of each run was used to reequilibrate the column prior to the next injection with a flow rate of 1 mL/min. Chromatographic peaks were identified using ultraviolet absorbance at 208 nm. Individual phospholipid peaks were identified by comparison of retention time to commercial standards. Phospholipid fractions were collected and stored at -80°C until later analysis for fatty acid content.

HPLC Materials—HPLC-grade hexane, water and potassium acetate were obtained from EMD Chemicals Inc. (Gibbstown, NJ), 2-propanol, acetonitrile and glacial acetic acid were obtained from J. T. Baker Inc. (Phillipsburg, NJ), ethanol was obtained from Pharmco products Inc. (Brookfield, CT). Phospholipid standards were obtained from Avanti Polar lipids (Alabaster, AL).

Transesterification and Gas Chromatography—The lipid fractions prepared by HPLC were transesterified as follows: 1 mL of hexane and 1 mL of 14% BF_3 -methanol was added and the mixture was incubated at 100°C for 1 h. The fatty acid methyl esters were extracted with hexane and analyzed by gas chromatography as described above. A pulsed splitless mode was used for GC analysis of methyl esters from phosphatidylinositol, phosphatidylserine and cardiolipin due to the low concentration of these fractions. GC methodology was as previously described [27] with the exception that ramp pressure was initially 55.5 psi with a 0.63 min hold; thereafter pressure was ramped at a rate of 50 psi/min to 14.50 psi, with a 60 min hold. The run time for a single sample was 74 min, with a sample injection-to injection time of 76 min.

Phospholipid Molecular Species Analysis of Cardiolipin

For phospholipid molecular species analysis, heart lipids were extracted in the presence of deuterium-labeled phospholipid standards and analyzed using reversed-phase high-performance liquid chromatography/electrospray ionization–mass spectrometry (HPLC/ESI–MS) with a C18 column (Prodigy, 150×2.0 mm, 5 μm ; Phenomenex, Torrance, CA) as described previously [29]. The separation was accomplished using a linear solvent gradient (water:0.5% ammonium hydroxide in methanol:hexane), changing from 12:88:0 to 0:88:12 in 17 min after holding the initial solvent composition for 3 min at a flow rate of 0.4 mL/min [30]. An Agilent 1100 LC/MSD instrument (Palo Alto, CA) was used to detect the separated phospholipid molecular species. For electrospray ionization, the drying gas temperature was 350°C ; the drying gas flow rate and nebulizing gas pressure were 11 L/min and 45 psi, respectively. The capillary and fragmentor voltages were set at 4,500 and 300 V, respectively. Identification of individual phospholipid molecular species was based on the monoglyceride, diglyceride and protonated molecular ion peaks [31].

Statistical Analysis

All data were expressed as the mean $\pm$ SEM. Mouse growth was tested using repeated measures ANOVA (Statsoft, Tulsa, OK). Tissue fatty acid comparisons at 20 weeks of age were performed using a one-way ANOVA. The analysis was followed by Tukey HSD post hoc test for determination of statistical significance among the three dietary groups.

Results

Body and Organ Weights of Dam-Reared and Artificially Reared Mice

The AR pups had a significantly lower body weight than that of dam-reared pups from PND 2 to PND 21; Fig. 1 illustrates changes in body weight in the three groups. There was no significant difference in body weight at PND 21 and the mean body weights were 10.68 ± 0.33 g for the n-3 Def, 10.73 ± 0.19 g for the n-3 Adq, and 12.12 ± 0.27 g for the dam-reared groups.

After PND 21, the AR pups gained weight rapidly so as to catch up and surpass the dam-reared pups. At 20 weeks of age, the mean body weights were 59.4 ± 1.5 g for the n-3 Def, 59.4 ± 1.0 g for the n-3 Adq, and 54.1 ± 1.4 g for the dam-reared groups. At 20 weeks of age there were no differences detected by one-way ANOVA in excised tissue weights between the AR and dam-reared groups except for the brain (the organ weights were for the brain (dam-reared group, 0.50 ± 0.01 g; n-3 Adq, 0.47 ± 0.01 g; n-3 Def, 0.460 ± 0.004 g), liver (dam-reared group, 2.66 ± 0.18 g; n-3 Adq, 3.13 ± 0.19 g; n-3 Def, 2.98 ± 0.24 g), heart (dam-reared group, 0.22 ± 0.01 g; n-3 Adq, 0.20 ± 0.01 g; n-3 Def, 0.21 ± 0.01 g), and retina (dam-reared group, 5.18 ± 0.42 mg; n-3 Adq, 5.64 ± 0.38 mg; n-3 Def, 5.43 ± 0.51 mg).

Fatty Acid Composition of Tissues

The 22:6n3 was significantly lower in the n-3 Def group as compared with the dam-reared and n-3 Adq groups in all tissues. In the n-3 Adq group, the 22:6n3 was similar to the dam-reared animals in all tissues, except liver. The decreased content of 22:6n3 for the n-3 Def group was largely replaced by a marked elevation in the percentage of 22:5n6 ($P < 0.0001$).

The 20:4n6 and 22:4n6 were also increased in the n-3 Def group compared with levels in the dam-reared and n-3 Adq groups except in heart tissue where there was no significant difference among the three dietary groups in the percentage of 22:4n6.

The brain, retina, and heart tissues had significantly lower docosapentaenoic acid (DPAn3; 22:5n3) in the n-3 Def group compared with the dam-reared and n-3 Adq groups whereas the plasma had significantly higher 22:5n3 in n-3 Def group compared with the dam-reared and n-3 Adq groups; the 22:5n3 was significantly different among the three dietary groups in the liver.

In the liver and retina, there were statistically significant differences in the ratio of 22:5n6/22:6n3 and n-6/n-3 between the dam-reared and n-3 Adq groups versus the n-3 Def group ($P < 0.0001$ for 22:5n6/22:6n3, $P < 0.0001$ for the n-6/n-3); for the liver, plasma and heart, there were significant differences in the ratio of 22:5n6/22:6n3 and n-6/n-3 in the n-3 Def group compared with those of the dam-reared and n-3 Adq groups ($P < 0.0001$ for 22:5n6/22:6n3, $P < 0.0001$ for n-6/n-3).

In brain, retina, heart and plasma the n-3 Def group showed a significantly higher percentage of total n-6 and a lower percentage of total n-3 compared with the n-3 Adq and the dam-reared group. In liver, the n-3 Def group showed a significantly lower percentage of total n-3 compared with those of the dam-reared, and n-3 Adq groups; there was no significant differences in total n-6 PUFA among the three dietary groups.

It must be noted that significant differences in weight percent do not necessary imply significant differences in absolute weight due to differences in some cases of absolute total fatty acid content. It should be noted in this regard that the absolute concentration of liver total fatty acid was significantly greater in the two controlled diets relative to the dam-reared control (Table 5) and this may suggest steatosis. This may be due to the very high content of saturated fatty acids (about 71–81%) in the two controlled diets due to the restriction in the use of vegetable oils in the basal fat that contained omega-3 fatty acids. Although the maternal diet also had a similarly high content of saturated fatty acids, the maternal milk composition could not be controlled in this regard. Since the two experimental diets (n-3 Adq and n-3 Def) had a similar liver total fatty acid content, the differences noted above in the weight percent data also correspond to differences in absolute concentrations.

When the results are presented on a relative percentage basis, the percentage of each fatty acid is influenced by changes in the other fatty acids. On the other hand, if the fatty acid results

were reported as absolute concentrations, changes in fatty acids would be independent of each other. Detailed fatty acid compositions, including total fatty acid concentrations of each dietary group for each tissue, are included in Tables 3, 4, 5, 6, and 7.

Heart Lipid Classes

Since heart total lipid exhibited an extremely high 22:6n3 level when dietary 22:6n3 was supplied, a percentage much higher than that observed in any other tissue, it was of interest to further delineate the lipid pools within which the 22:6n3 resided. A non-uniform distribution of the major phospholipid classes in cardiac muscle has been reported [32]. Both the distribution of the major heart phospholipid classes, as well as their fatty acid composition was determined, in order to more clearly define the extent of the diet-induced changes. Significant changes in the distribution of phosphatidylcholine ($P < 0.01$), phosphatidylethanolamine ($P < 0.05$), neutral lipid ($P < 0.05$), cardiolipin ($P < 0.001$) and phosphatidylinositol ($P < 0.01$) were observed between the dietary groups (Table 8). The amount of the remaining material was below the level required for accurate determination of other lipid classes with the method employed.

When examining the fatty acid composition across the phospholipid classes under investigation, we find that, in general, there was no effect of the diet upon the distribution of the major saturated fatty acids (palmitic acid, 16:0; stearic acid, 18:0). 22:6n3 was concentrated in the phosphatidylethanolamine, phosphatidylcholine and cardiolipin fractions reaching 40, 29 and 15%, respectively, of total fatty acids in diets containing preformed 22:6n3. Again 22:5n6 replaced 22:6n3 in the n-3 Def case, and also 20:4n6 was very significantly elevated in all phospholipid classes relative to the n-3 Adq and dam-reared groups. While cardiac cardiolipin makes up only about 6–8% of the total phospholipid of cardiac muscle membranes, it contains a very high proportion of 18:2n6 compared with other phospholipid classes (See Tables 9 and 10).

In further investigation of the reservoirs for 22:6n3, a molecular species analysis of cardiolipins was performed. It was observed, for the first time, that the heart cardiolipin in the 22:6n3-supplemented group contained molecular species with four 22:6n3 moieties. HPLC/EIS-MS analysis of heart phospholipids indicated that cardiolipin in the n-3 Adq dietary group contained abundant 22:6 species, including 22:6,22:6/22:6,22:6, 22:6,22:6/18:2,22:6, 18:2, 22:6/18:2,22:6, 18:2,22:6/18:2,18:2, 18:2,22:6/18:2,18:1, 18:2,18:1/18:1,22:6, and 18:1,18:1/18:2,22:6 species. These assignments were based on the diglyceride ions detected at m/z 600, 648, 602, 650 and 696, representing 18:2,18:2 (di-linoleoyl), 18:2,22:6 (linoleoyl, docosahexaenoyl), 18:1,18:2 (oleoyl, linoleoyl), 18:1,22:6 (oleoyl, docosahexaenoyl) and 22:6,22:6 (di-docosahexaenoyl) species, respectively. Considering that cardiolipin contains four fatty acyl chains, two diglyceride fragment peaks overlapping in the ion chromatogram indicated the presence of these cardiolipin molecular species, although positional information cannot be obtained using this approach. In contrast, in the n-3 Def diet group, 18:2,18:2/18:2,18:2 is the predominant molecular species in cardiolipin, with the 22:5-containing species 18:2,18:2/18:2,22:5 and 18:2,18:2/16:0,22:5 species as rather minor species. No 22:5,22:5 ion at m/z 700 was detected in the cardiolipin. These data suggested that 22:6n3 may be the preferred fatty acid incorporated into cardiolipin in comparison to 22:5n6.

Discussion

The AR technique has provided nutritionists with a powerful research tool. The main advantage of the AR technique is that it allows precise control of the amount and composition of milk-substitute that each pup receives. Historically, the species most studied using the AR technique is the rat. In addition to the obvious cost advantages of feeding and housing a smaller animal, the rat has emerged as a species of choice for these studies because of its well-defined

nutritional requirements and long history of use in metabolic studies. The relatively large number of pups in a rodent litter also enables control and experimental animals to be chosen from the same litters. Finally, the small size of the rat makes its rearing apparatus easy to design and assemble. This work established that the Hoshiba [4] artificial rearing system could be applied to the mouse for studies of essential fatty acid nutrition throughout the neonatal period.

The body weight of the mice in the present study has the same pattern as reported previously [5,33]; that is the AR groups showed lower weight gain than the dam-reared group until the time of weaning. No significant differences were detected between the growths of the two experimental diet groups. It should be noted that body weights were not reported beyond the weaning period in the above mentioned studies, hence comparison is not possible. From the later observation, it appears that the AR procedures may have induced some difference in ad libitum feeding behavior.

In our study, the mortality of pups was 12% in the artificially reared groups. Three pups in the n-3 Def group and one pup in the n-3 Adq group died after aspirating milk into the lungs. Different sizes of nipples were used that better fit the mouth of the pups as they aged so as to avoid bloating that can occur due to air aspiration through the gap between the incisors and nipple as we noted that this can lead to pup mortality.

Although the AR technique has several advantages, some limitations do exist, although future refinements will likely overcome these. One such limitation is that it eliminates many of the maternal interactions experienced by naturally reared pups. Lack of these interactions may have profound effects on brain and behavioral development. Also, the presence or absence of some unknown factor(s) in the milk-substitute formula that are present in mother's milk may promote normal organ development, especially of the intestine [21,34].

Other studies have used a mouse model for feeding a diet deficient in n-3 fatty acids including three [35]; two [36,37]; and one generation models [38]. Because of the increasing availability of tools for genetic manipulation [39,40], the mouse has become the most popular animal model; development of this technique will provide a means to perform studies involving nutritional manipulation of essential fatty acids in neonatal mice in conjunction with genetically derived enzymatic modifications of interest including desaturase knock out [41], fat1 [39] and SCD-1 [42] deficiency. In addition, it should be clear that any nutrient whose content can be controlled in the artificial milk can provide an independent variable for an AR experiment and so there should be many applications of this method for nutritional studies.

It was important initially to establish whether this "one generation" model can generate large enough losses in the nervous system as well as other organ levels of 22:6n3 so that functional changes can be observed. It must first be considered what the extent of brain/retinal DHA losses are necessary for behavioral/physiological parameters to be significantly altered. Lim et al. [43] have shown that adult rats with a 70% loss in brain 22:6n3 performed more poorly in spatial tasks. Weisinger et al. [44] showed that there was a loss in retinal sensitivity and b-wave implicit times in rats after three generations of n-3 deficiency. However, these rats had only a 55% loss of retinal 22:6n3, and showed no differences in α -wave amplitudes or in most of the phototransduction parameters measured. Moriguchi and Salem [45] have demonstrated, in a 22:6n3 repletion paradigm, that spatial task performance is altered when the brain level of 22:6n3 declines by 40% or more. Thus, a one generation model of n-3 fatty acid deficiency that better mimics the human situation but that still produces a substantial decline in brain and retinal 22:6n3 of 40-50% or more would be very valuable for the development of this field.

This method was shown to be an efficient means for inducing brain and peripheral organ n-3 fatty acid deficiency as the pups fed the n-3 Def formula exhibited a loss of 51% of their brain 22:6n3 by 20 weeks of age, replacing it primarily with 22:5n6. These changes were consistent

with the proposal by Salem et al. [46] that reciprocal replacement of 22:6n3 with 22:5n6 should be extended to include other n-6 fatty acids like 20:4n6 and 22:4n6 since for the case of the retina and brain, only when both 22:5n6 and 22:4n6 were summed together with 22:6n3 was reciprocal replacement of 22:6n3 complete at all time points when comparing n-3 fatty acid adequate and deficient diets. The liver, heart, plasma, and the retinal percentages of 22:6n3 were 91, 90, 86 and 29% lower, respectively, in the pups fed the n-3 Def milk formula relative to those fed the same formula to which 3.1% of 18:3n3 and 0.93% of 22:6n3 had been added. As expected then, the peripheral organ losses in 22:6n3 and other n-3 fatty acids was much more drastic than those observed in the nervous system. Thus, the present feeding method results in nutritional deficiencies comparable to those observed in multiple generational models and should prove useful for studies of 22:6n3 function in the nervous system as well as peripheral organs.

It must be noted here that the percentage of 22:6n3 in the n-3 Adq group in the heart reached 31%, a level much greater than that of the brain or retina, prompting us to investigate in which lipid classes the 22:6n3 was concentrated. Our data indicate that phosphatidylethanolamine and phosphatidylcholine were the major repositories of 22:6n3 in the mouse heart, with 22:6n3 contents of 40 and 29%, respectively. In a review by Wessels and Sedmera [47]; the authors concluded that, apart from the obvious differences in size, the mouse and human heart are anatomically remarkably similar throughout development. They also concluded that employing the mouse as a model system for the human heart is useful. This extremely high level of heart 22:6n3 in a diet containing 22:6n3 has not been previously noted, to our knowledge. It is also noteworthy that the level of 22:6n3 provided in our diet was less than 1 wt % of fatty acids, a level obtained in many modern diets. Perhaps the critical factor leading to a remarkably high 22:6n3 content in mouse heart is the feeding from the very beginning of life where fatty acid compositions are more malleable.

When comparing the level of 22:6n3 in the mouse heart versus that of the rat, the 22:6n3 content of the rat heart is much lower than the mouse heart reaching 10% for a diet containing only 18:3n3 (3.1% of 18:3n3 and no 22:6n3) and 15% for a diet containing 2.6% of 18:3n3 plus 1.3% of 22:6n3 (unpublished data). This then prompts the question of what this apparent species difference may be due to. The underlying differences in metabolism leading to this very significant response to dietary 22:6n3 between these species is unclear but it is noted that the murine heart has an elevated heart rate of 500–700 beats/min, whereas the rat has a heart rate of 300–400 beats/min [48]. It has previously been reported that 22:6n3 is concentrated in tissues, such as the hummingbird flight muscles, that have extremely high metabolic activity [49].

Observational, clinical, and experimental studies have demonstrated that long-chain n-3 PUFA supplementation reduces the risk of heart disease in humans including cardiac arrhythmias [50,51]. In n-3 depleted rats, it was observed that within 2 h after intravenous bolus injection with medium chain-triacylglycerols: fish oil, the emulsion modifies several cationic events likely to be involved in contractile functions of aortic ring preparations [52]. A positive association between levels of circulating n-3 fatty acids derived from normal dietary variation and endothelial function was shown in young adults who smoked or had higher levels of fasting insulin, glucose or triglycerides. Given the central position of the endothelium in early atherogenesis, a protective influence of 22:6n3 may, in part, explain the epidemiological association between increased fish intake and reduced cardiovascular mortality and morbidity [53].

In a recent study by Garg et al. [17]; the authors demonstrated that short-term supplementation with a fish oil concentrate results in increased levels of 20:5n3 and 22:6n3, whereas the levels of the eicosanoid substrate, 20:4n6 decreased in the human atrium. However, more 22:6n3 than

20:5n3 is incorporated in the atrium total lipids (22:6n3/20:5n3 ratio of 2.54) and phospholipids (22:6n3/20:5n3 ratio of 2.78) despite the fish oil supplement supplying a higher amount of 20:5n3 than 22:6n3. The author also concluded that the enrichment of the phospholipid fraction with long-chain n-3 PUFA suggests that these fatty acids were readily available for release as non-esterified acids should an arrhythmic event occur. The accumulation of 20:5n3 and 22:6n3 was also observed during fish oil supplementation in myocardial fatty acids in humans [54] and that was at the expense of 20:4n6; 22:6n3 accumulated in atrial phospholipids more rapidly than did 20:5n3, even though 22:6n3 and 20:5n3 were present in equal proportions in the fish oil supplement used. The rapid accumulation of 22:6n3 was also reported in rat cardiac phospholipids without a similar increase in heart 20:5n3 content [13], and it was also confirmed in this mouse study; suggesting a specific mechanism for the uptake and/or retention of 22:6n3. The content of 20:5n3 in mouse cardiac muscle phospholipids was very low. This was apparent in both the total phospholipids (Table 7) and in all the major phospholipid classes examined in detail (Table 9). In the present study, consistent with these reports, the 20:5n3 content was very low in all tissues studied when dietary sources of 18:3n3 and 22:6n3 were provided.

In mammals, cardiolipin may contain up to 85 wt % of fatty acids as 18:2n6. However, this composition is known to be malleable as when the influence of maternal dietary fatty acids on the acyl composition of offspring cardiolipin was examined, feeding fish oil resulted in replacement of a substantial portion of 18:2n6 with 22:6n3 in 42-day-old mice concomitant with a reduction in 18:2n6 from 62% (safflower oil fed) to 12% [55]. In the present study, the 18:2n6 content of the cardiolipin was 63% in the n-3 Def group and 41% in the n-3 Adq group. Watkins et al. [56] reported that cardiolipin did not accumulate more 22:6n3 than other phospholipid classes, and the mass of cardiolipin per g of heart did not change in response to a crocodile oil diet (containing 3% 22:6n3 and 1% 20:5n3) fed to weanling, 21-day-old mice until the 112th day of age. Heart phospholipids were enriched with the metabolic products of 18:3n3 in mice fed soybean oil suggesting that either 18:3n3 was converted to 22:6n3 within the heart itself or that the heart preferentially accumulated fatty acids from a plasma lipid pool enriched in 22:6n3. A novel observation in the present study was that in mice fed preformed 22:6n3, heart cardiolipin contained species with three and four 22:6n3 moieties. The occurrence of these rather extraordinary species has never been previously observed, to our knowledge. Together with phosphatidylcholine and phosphatidylethanolamine, the cardiolipin forms a repository for a high degree of 22:6n3 accretion in the mouse heart. This study lays the groundwork for studies of the unique biophysical role of tetra-22:6n3-cardiolipin species.

In conclusion, this study demonstrates the rather remarkable extent to which tissue fatty acid composition of internal organs, particularly the heart, but also nervous system tissues can be modified when the dietary fatty acid intake is controlled from the first days of life. The AR method employed here should be a boon to the study of EFA function, especially when combined with suitable genetically modified lines of mice. For example, desaturase knockout mice can be reared with or without particular essential fatty acids so that the relationship to physiological functions can be understood. It was notable that the mouse appeared to be a very responsive species in its organ fatty acid composition with respect to a diet containing preformed 22:6n3. Mouse heart phospholipids incorporated a very high level of 22:6n3 and a species of cardiolipin containing four 22:6n3 molecules were described for the first time.

Abbreviations

AR

Artificial rearing

n-3 Adq

n-3 Fatty acid adequate group

n-3 Def**n-3 Fatty acid deficient group****References**

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Fig. 1. Growth curves of artificially reared and dam-reared mouse pups from PND2 through week 20. The body weight is presented as the mean $\pm$ SEM, with an $n = 14$ or 16. Asterisk indicates $P < 0.001$ for Dam's group versus the artificially reared groups from PND2–PND21

Table 1

Nutrient and fatty acid composition of pelleted diets given to dams and pups at weaning

Ingredient	Amount (g/100 g)		
Casein, vitamin free ^a	20		
Carbohydrate	60		
Corn starch	15		
Sucrose	10		
Dextrose	19.9		
Maltose-dextrin	15		
Cellulose	5		
Mineral and salt mix ^b	3.5		
Vitamin mix ^c	1		
L-Cystine	0.3		
Choline bitartrate	0.25		
TBHQ ^d	0.002		
Fat	Maternal	n-3 Def	n-3 Adq
Hydrogenated coconut oil	7.75	8.1	7.45
Safflower oil	1.77	1.9	1.77
Flaxseed oil	0.48	–	0.48
DHASCO ^e	–	–	0.3
Fatty acid composition (wt %)			
Σ Saturated	74.6	80.9	71.1
Σ Monounsaturated	5.1	3.5	6.0
18:2n6	15.3	13.4	16.0
18:3n3	3.1	0.09	2.9
22:6n3	–	–	1.6
n-6/n-3	5.8	143	3.5

^aDyets Inc. catalogue #400625 for maternal diet and ALACID casein (NZMP North America Inc) for n-3 Def and n-3 Adq diet^bDyets Inc. catalogue #210025^cDyets Inc. catalogue #310025^dTBHQ is tert-butyl hydroquinone^eMartek Biosciences Corporation. DHASCO refers to a mixture of oil extracted from the unicellular algae *Cryptocodinium cohnii* and high oleic sunflower oil, the resulting mixed oil contains 44% of product weight as 22:6n3

Table 2

Nutrient composition of the artificial milk for mouse pups

Ingredient	Amount (weight/100 mL milk)	
	n-3 Def	n-3 Adq
Protein (g)		
Whey protein (Alacen, WPI) ^a	3.64	3.64
Whey protein Hydrolysate ^g	4.00	4.00
Casein (Alacid, acid casein) ^a	3.46	3.46
Carbohydrate (g)		
alpha-Lactose ^b	2.6	2.6
Fat (g)		
MCT oil ^d	2.08	2.08
Coconut oil (hydrogenated) ^e	4.32	4.32
18:1n9 ethyl ester ^f	7.2	6.6
18:2n6 ethyl ester ^f	2.4	2.4
18:3n3 ethyl ester ^f	—	0.48
22:6n3 ethyl ester ^f	—	0.16
Cholesterol ^b	0.04	0.04
Vitamins (g)		
Vitamin mix ^c	0.50	0.50
Vitamin C ^b	0.02	0.02
Tricholine citrate ^b	0.37	0.37
Minerals (mg)		
NaOH ^b	102.5	102.5
KOH ^b	170	170
GlyCaPO ₄ ^b	800	800
MgCl ₂ 6H ₂ O ^b	183	183
CaCl ₂ 2H ₂ O ^b	210	210
Ca ₃ -4H ₂ O-citrate ^b	250	250
Na ₂ HPO ₄ ^b	114	114
KH ₂ PO ₄ ^b	51	51
FeSO ₄ ^b	3.0	3.0
ZnSO ₄ ^b	6.0	6.0
CuSO ₄ ^b	1.6	1.6
MnSO ₄ ^b	0.075	0.075
NaF ^b	0.15	0.15
KI ^b	0.18	0.18
K ₂ SO ₄ ^b	163.1	163.1
Na ₂ SeO ₄ ^b	0.036	0.036
(NH ₄) ₂ MoO ₄ ^b	0.028	0.028
Na ₂ O ₃ Si 9H ₂ O ^b	5.075	5.075
CrK(SO ₄) ₂ 12H ₂ O ^b	0.963	0.963
LiCl ^b	0.061	0.061
H ₃ BO ₃ ^b	0.285	0.285
NiCO ₃ ^b	0.111	0.111
NH ₄ VO ₃ ^b	0.023	0.023
Others (g)		
Carnitine ^b	0.004	0.004
Picolinate ^b	0.002	0.002
Ethanolamine ^b	0.004	0.004
Taurine ^b	0.015	0.015
Serine ^b	0.03	0.03
Cystine ^b	0.3	0.3
Tryptophan ^b	0.03	0.03
Methionine ^b	0.005	0.005
Actual fatty acid composition of artificial mouse milk (wt %) ^h		
Σ Saturated	30.62	32.62
Σ Monounsaturated	49.09	45.79
18:2n6	16.72	16.9
18:3n3	0.06	3.14
22:6n3	—	0.93
n-6/n-3	207.6	4.2

Component sources were as follows:

^aNZMP (North America) Inc, Santa Rosa, CA;

^bSigma-Aldrich Corp. St. Louis, MO;

^cHarlan, Madison, WI;

^dNovartis Medical Nutrition, Fremont, MI;

^eDyets, Bethlehem, PA;

^fNu-Chek Prep. Inc. Elysian, MN;

^gChicago Sweeteners, Chicago, IL

^hThis fatty acid profile was analyzed by transmethylation of each artificial milk and GC analysis. Only trace quantities of the long-chain polyunsaturated fatty acids 20:4n6, 20:5n3, 22:5n3 were detected (i.e., <0.01%). Other minor peaks were not included

Table 3

Brain fatty acid composition of the two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

	Dietary group		
	Dam-reared (n = 16)	n-3 Adq (n = 14)	n-3 Def (n = 14)
Fatty acids			
12:0	0.021 ± 0.002 ^a	0.028 ± 0.002 ^b	0.020 ± 0.002 ^a
14:0	0.94 ± 0.03 ^a	0.91 ± 0.03 ^a	0.78 ± 0.03 ^b
16:0 DMA	0.04 ± 0.01 ^a	ND ^a	0.52 ± 0.08 ^b
16:0	19.67 ± 0.14	20.19 ± 0.27	19.89 ± 0.22
18:0 DMA	ND	ND	0.22 ± 0.07
18:0	18.17 ± 0.14 ^a	18.99 ± 0.33 ^b	18.49 ± 0.11 ^{ab}
20:0	0.39 ± 0.01	0.35 ± 0.02	0.36 ± 0.02
22:0	0.51 ± 0.02 ^a	0.46 ± 0.02 ^a	0.62 ± 0.03 ^b
24:0	1.08 ± 0.07	0.99 ± 0.11	0.90 ± 0.05
Total saturates	40.83 ± 0.20	41.94 ± 0.56	41.81 ± 0.25
16:1n7	0.95 ± 0.06	0.88 ± 0.07	0.86 ± 0.05
18:1 DMA	ND	ND	0.13 ± 0.03
18:1n9	15.38 ± 0.16 ^a	14.17 ± 0.33 ^b	13.70 ± 0.15 ^b
18:1n7	3.71 ± 0.04 ^a	3.42 ± 0.07 ^b	3.82 ± 0.04 ^a
20:1n9	2.19 ± 0.08 ^a	1.81 ± 0.12 ^b	1.81 ± 0.09 ^b
22:1n9	0.28 ± 0.01 ^a	0.22 ± 0.01 ^b	0.23 ± 0.02 ^b
24:1n9	3.29 ± 0.11	2.91 ± 0.22	2.82 ± 0.11
Total monounsaturates	25.80 ± 0.39 ^a	23.41 ± 0.74 ^b	23.37 ± 0.40 ^b
18:2n6	0.53 ± 0.01 ^a	0.50 ± 0.02 ^a	0.37 ± 0.02 ^b
18:3n6	ND	ND	ND
20:2n6	0.131 ± 0.004 ^a	0.13 ± 0.01 ^a	0.10 ± 0.01 ^b
20:3n6	0.55 ± 0.01 ^a	0.54 ± 0.01 ^a	0.25 ± 0.01 ^b
20:4n6	7.06 ± 0.12 ^a	7.85 ± 0.16 ^b	9.01 ± 0.14 ^c
22:2n6	ND	ND	ND
22:4n6	2.19 ± 0.05 ^a	2.43 ± 0.04 ^b	3.68 ± 0.04 ^c
22:5n6	0.19 ± 0.01 ^a	0.25 ± 0.01 ^a	6.45 ± 0.19 ^b
Total n-6 PUFA	10.65 ± 0.14 ^a	11.70 ± 0.17 ^b	19.86 ± 0.26 ^c
18:3n3	0.060 ± 0.003 ^a	0.061 ± 0.004 ^a	0.041 ± 0.003 ^b
20:5n3	0.090 ± 0.004 ^a	0.08 ± 0.01 ^b	ND ^c
22:5n3	0.26 ± 0.01 ^a	0.23 ± 0.01 ^a	0.10 ± 0.02 ^b
22:6n3	14.57 ± 0.32 ^a	15.70 ± 0.35 ^b	7.74 ± 0.20 ^c
Total n-3 PUFA	14.97 ± 0.32 ^a	16.07 ± 0.36 ^b	7.88 ± 0.20 ^c
16:1n7/16:0	0.05 ± 0.003	0.04 ± 0.004	0.04 ± 0.003
18:1n9/18:0	0.85 ± 0.01 ^b	0.75 ± 0.03 ^a	0.74 ± 0.01 ^a
20:4n6/20:3n6	12.94 ± 0.40 ^b	14.70 ± 0.35 ^b	36.17 ± 1.30 ^a
20:4n6/20:5n3	81.12 ± 4.24	109.54 ± 6.52	ND
20:4n6/22:6n3	0.49 ± 0.01 ^b	0.50 ± 0.01 ^b	1.17 ± 0.03 ^a
20:5n3/22:6n3	0.01 ± 0.0003	0.005 ± 0.0004	ND
22:5n6/22:6n3	0.013 ± 0.001 ^a	0.016 ± 0.001 ^a	0.84 ± 0.03 ^b
22:5n6+22:6n3	14.75 ± 0.31 ^a	15.95 ± 0.35 ^b	14.19 ± 0.26 ^a
n-6/n-3	0.71 ± 0.01 ^a	0.73 ± 0.01 ^a	2.54 ± 0.07 ^b
n-6+n-3	25.62 ± 0.42 ^a	27.76 ± 0.50 ^b	27.74 ± 0.36 ^b
Total fatty acids (µg/mg wet brain)	41.26 ± 0.46 ^a	39.16 ± 0.49 ^b	40.99 ± 0.44 ^a

Each parameter is presented as the mean (±SEM). Means with different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$

ND not detected (i.e., <0.01%), DMA dimethyl acetal

Table 4

Retina fatty acid composition of the two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

	Dietary group		
	Dam-reared (n = 16)	n-3 Adq (n = 14)	n-3 Def (n = 14)
Fatty acids			
10:0	0.13 ± 0.02 ^a	0.26 ± 0.06 ^{ab}	0.36 ± 0.03 ^b
12:0	0.052 ± 0.005 ^a	0.10 ± 0.01 ^b	0.09 ± 0.01 ^{ab}
14:0	1.14 ± 0.06 ^a	1.30 ± 0.07 ^a	0.46 ± 0.02 ^b
16:0	19.78 ± 0.35 ^a	20.72 ± 0.50 ^a	18.44 ± 0.15 ^b
18:0	17.11 ± 0.37	17.08 ± 0.40	18.01 ± 0.28
20:0	0.23 ± 0.01 ^{ab}	0.25 ± 0.01 ^a	0.21 ± 0.01 ^b
22:0	0.16 ± 0.01 ^a	0.19 ± 0.02 ^a	0.12 ± 0.01 ^b
24:0	0.13 ± 0.01 ^a	0.14 ± 0.02 ^a	0.23 ± 0.01 ^b
Total saturates	38.72 ± 0.62 ^{ab}	40.05 ± 0.63 ^a	37.92 ± 0.21 ^b
12:1	0.25 ± 0.09	0.18 ± 0.06	0.18 ± 0.05
14:1	0.27 ± 0.06	0.65 ± 0.20	0.67 ± 0.08
16:1n7	0.69 ± 0.06 ^{ab}	0.89 ± 0.10 ^a	0.55 ± 0.06 ^b
18:1n7	1.54 ± 0.06 ^a	1.71 ± 0.06 ^a	1.97 ± 0.05 ^b
18:1n9	10.51 ± 0.59	11.39 ± 0.57	9.76 ± 0.38
20:1n9	0.64 ± 0.06 ^a	0.50 ± 0.06 ^{ab}	0.36 ± 0.03 ^b
22:1n9	0.03 ± 0.01	0.04 ± 0.01	0.027 ± 0.002
24:1n9	0.20 ± 0.02	0.27 ± 0.04	0.21 ± 0.02
Total monounsaturates	14.14 ± 0.66	15.64 ± 0.75	13.73 ± 0.47
18:2n6	0.69 ± 0.03 ^a	0.84 ± 0.07 ^b	0.45 ± 0.02 ^c
18:3n6	0.022 ± 0.001 ^a	0.07 ± 0.03 ^b	0.048 ± 0.003 ^{ab}
20:2n6	0.10 ± 0.01	0.16 ± 0.05	0.13 ± 0.01
20:3n6	0.38 ± 0.01 ^a	0.37 ± 0.02 ^a	0.26 ± 0.02 ^b
20:4n6	7.17 ± 0.21 ^a	7.93 ± 0.21 ^b	9.86 ± 0.15 ^c
22:2n6	0.025 ± 0.003 ^a	0.04 ± 0.01 ^{ab}	0.05 ± 0.01 ^b
22:4n6	0.84 ± 0.04 ^a	0.96 ± 0.03 ^a	2.44 ± 0.10 ^b
22:5n6	0.11 ± 0.01 ^a	0.13 ± 0.01 ^a	5.72 ± 0.66 ^b
Total n-6 PUFA	9.33 ± 0.24 ^a	10.50 ± 0.21 ^a	18.96 ± 0.79 ^b
18:3n3	0.018 ± 0.003 ^a	0.033 ± 0.004 ^b	0.046 ± 0.004 ^c
20:3n3	0.025 ± 0.003	0.028 ± 0.004	0.03 ± 0.01
20:5n3	0.47 ± 0.03 ^a	0.39 ± 0.03 ^b	0.017 ± 0.002 ^c
22:5n3	0.67 ± 0.02 ^a	0.63 ± 0.03 ^a	0.27 ± 0.02 ^b
22:6n3	18.91 ± 1.41 ^a	18.31 ± 1.58 ^a	12.95 ± 0.78 ^b
Total n-3 PUFA	20.09 ± 1.39 ^a	19.38 ± 1.57 ^a	13.32 ± 0.78 ^b
16:1n7/16:0	0.03 ± 0.003	0.04 ± 0.005	0.03 ± 0.003
18:1n9/18:0	0.62 ± 0.04 ^{ab}	0.67 ± 0.04 ^a	0.55 ± 0.03 ^b
20:4n6/20:3n6	19.23 ± 0.79 ^a	21.84 ± 1.05 ^a	40.49 ± 3.48 ^b
20:4n6/20:5n3	15.99 ± 1.04 ^a	21.55 ± 1.32 ^a	661.14 ± 64.04 ^b
20:4n6/22:6n3	0.41 ± 0.03 ^a	0.48 ± 0.04 ^a	0.81 ± 0.06 ^b
20:5n3/22:6n3	0.03 ± 0.003 ^a	0.02 ± 0.003 ^a	0.001 ± 0.0002 ^b
22:5n6/22:6n3	0.007 ± 0.001 ^a	0.008 ± 0.001 ^a	0.43 ± 0.03 ^b
22:5n6+22:6n3	19.02 ± 1.41	18.44 ± 1.58	18.66 ± 1.39
n-6/n-3	0.46 ± 0.03 ^a	0.54 ± 0.05 ^a	1.42 ± 0.05 ^b
n-6+n-3	29.42 ± 1.42	29.89 ± 1.54	32.28 ± 1.50
Total fatty acids (µg/mg wet retina)	23.72 ± 2.13 ^b	19.63 ± 1.76 ^b	36.78 ± 4.02 ^a

* Each parameter is presented as the mean (± SEM). Means with different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$

Table 5

Liver fatty acid composition of the two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

	Dietary group		
	Dam-reared (n = 16)	n-3 Adq (n = 14)	n-3 Def (n = 14)
Fatty acids			
10:0	0.014 ± 0.002	0.013 ± 0.01	0.011 ± 0.001
12:0	0.32 ± 0.03	0.25 ± 0.01	0.31 ± 0.02
14:0	1.38 ± 0.05 ^{ab}	1.24 ± 0.05 ^a	1.56 ± 0.10 ^b
16:0	24.56 ± 0.35	24.13 ± 0.51	23.98 ± 0.54
18:0	5.90 ± 0.30 ^a	4.24 ± 0.43 ^b	4.47 ± 0.57 ^{ab}
20:0	0.41 ± 0.03 ^a	0.26 ± 0.03 ^b	0.22 ± 0.02 ^b
22:0	0.29 ± 0.02 ^a	0.16 ± 0.03 ^b	0.13 ± 0.02 ^b
24:0	0.033 ± 0.004 ^a	0.05 ± 0.02 ^{ab}	0.09 ± 0.02 ^b
Total saturates	32.91 ± 0.38 ^a	30.35 ± 0.52 ^b	30.77 ± 0.43 ^b
12:1	0.010 ± 0.002	0.011 ± 0.01	0.009 ± 0.001
14:1	0.09 ± 0.01	0.08 ± 0.01	0.14 ± 0.01
16:1n7	4.10 ± 0.16 ^a	4.58 ± 0.35 ^a	4.68 ± 0.30 ^b
18:1n7	4.66 ± 0.27 ^a	5.38 ± 0.24 ^a	7.35 ± 0.33 ^b
18:1n9	26.69 ± 1.68 ^a	35.92 ± 1.55 ^a	34.35 ± 2.14 ^b
20:1n9	0.85 ± 0.04 ^a	0.99 ± 0.04 ^b	0.99 ± 0.03 ^b
22:1n9	0.100 ± 0.005	0.09 ± 0.01	0.08 ± 0.01
24:1n9	0.20 ± 0.01	0.14 ± 0.02	0.15 ± 0.04
Total monounsaturates	36.70 ± 1.96 ^a	47.21 ± 1.88 ^b	47.74 ± 2.25 ^b
18:2n6	10.89 ± 0.80 ^a	8.53 ± 0.83 ^{ab}	7.57 ± 0.79 ^b
18:3n6	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
20:2n6	0.21 ± 0.01	0.17 ± 0.02	0.16 ± 0.03
20:3n6	1.05 ± 0.05 ^a	0.75 ± 0.07 ^b	0.73 ± 0.11 ^b
20:4n6	3.21 ± 0.21 ^a	2.50 ± 0.35 ^a	5.33 ± 0.88 ^b
22:2n6	0.021 ± 0.003 ^a	0.02 ± 0.01 ^a	0.051 ± 0.004 ^b
22:4n6	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.20 ± 0.04 ^b
22:5n6	0.048 ± 0.004 ^a	0.05 ± 0.01 ^a	1.32 ± 0.24 ^b
Total n-6 PUFA	15.63 ± 0.96	12.19 ± 1.11	15.46 ± 1.95
18:3n3	0.60 ± 0.06 ^a	0.53 ± 0.07 ^a	0.054 ± 0.004 ^b
20:3n3	0.060 ± 0.004 ^a	0.05 ± 0.01 ^{ab}	0.038 ± 0.004 ^a
20:5n3	0.68 ± 0.05 ^a	0.35 ± 0.04 ^b	0.025 ± 0.002 ^c
22:5n3	0.42 ± 0.05 ^a	0.26 ± 0.03 ^b	0.017 ± 0.004 ^c
22:6n3	7.70 ± 0.66 ^a	4.32 ± 0.50 ^b	0.38 ± 0.06 ^c
Total n-3 PUFA	9.46 ± 0.79 ^a	5.50 ± 0.60 ^b	0.51 ± 0.07 ^c
16:1n7/16:0	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
18:1n9/18:0	4.94 ± 0.58 ^b	9.77 ± 1.07 ^a	8.95 ± 0.93 ^a
20:4n6/20:3n6	3.06 ± 0.11 ^a	3.26 ± 0.22 ^a	7.29 ± 0.43 ^b
20:4n6/20:5n3	5.03 ± 0.43 ^a	8.05 ± 1.21 ^a	217.77 ± 28.19 ^b
20:4n6/22:6n3	0.44 ± 0.03 ^a	0.59 ± 0.04 ^a	14.05 ± 0.28 ^b
20:5n3/22:6n3	0.09 ± 0.004	0.08 ± 0.01	0.08 ± 0.01
22:5n6/22:6n3	0.007 ± 0.001 ^a	0.011 ± 0.001 ^a	3.39 ± 0.18 ^b
22:5n6+22:6n3	7.75 ± 0.67 ^a	4.37 ± 0.51 ^b	1.70 ± 0.30 ^c
n-6/n-3	1.65 ± 0.07 ^a	2.22 ± 0.08 ^a	30.17 ± 0.91 ^b
n-6+n-3	25.09 ± 1.73 ^a	17.69 ± 1.70 ^b	15.98 ± 2.02 ^b
Total fatty acids (µg/mg wet liver)	49.69 ± 3.07 ^b	86.06 ± 9.85 ^a	78.72 ± 7.26 ^a

Each parameter is presented as the mean (±SEM). Means with different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$

Table 6

Plasma fatty acid composition of the two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

	Dietary group		
	Dam-reared (n = 16)	n-3 Adq (n = 14)	n-3 Def (n = 14)
Fatty acids			
10:0	0.21 ± 0.02 ^a	0.11 ± 0.02 ^b	0.08 ± 0.01 ^b
12:0	3.69 ± 0.32 ^a	2.06 ± 0.33 ^b	1.07 ± 0.16 ^b
14:0	2.73 ± 0.19 ^a	1.64 ± 0.18 ^b	1.06 ± 0.11 ^b
16:0	18.27 ± 0.20 ^a	17.95 ± 0.21 ^a	14.77 ± 0.44 ^b
18:0	9.22 ± 0.16	9.05 ± 0.20	8.82 ± 0.31
20:0	0.37 ± 0.02 ^a	0.25 ± 0.02 ^b	0.19 ± 0.01 ^b
22:0	0.37 ± 0.02 ^{ab}	0.30 ± 0.01 ^a	0.48 ± 0.06 ^b
24:0	0.16 ± 0.01 ^a	0.14 ± 0.01 ^a	0.29 ± 0.03 ^b
Total saturates	35.01 ± 0.55 ^a	31.50 ± 0.68 ^b	26.75 ± 0.75 ^c
12:1	0.03 ± 0.01	0.022 ± 0.001	0.032 ± 0.004
14:1	0.06 ± 0.01 ^a	0.036 ± 0.004 ^b	0.049 ± 0.005 ^{ab}
16:1n7	2.84 ± 0.12	2.64 ± 0.18	2.42 ± 0.12
18:1n7	2.63 ± 0.20 ^a	2.85 ± 0.21 ^a	3.71 ± 0.19 ^b
18:1n9	14.65 ± 0.77 ^{ab}	16.30 ± 0.75 ^a	13.03 ± 0.41 ^b
20:1n9	0.61 ± 0.02 ^a	0.53 ± 0.03 ^{ab}	0.49 ± 0.03 ^b
22:1n9	0.12 ± 0.01 ^a	0.10 ± 0.01 ^a	0.39 ± 0.09 ^b
24:1n9	0.32 ± 0.01 ^a	0.41 ± 0.03 ^a	0.82 ± 0.07 ^b
Total monounsaturates	21.26 ± 0.96	22.89 ± 0.95	20.94 ± 0.52
18:2n6	22.54 ± 0.73 ^a	20.36 ± 0.76 ^a	16.53 ± 0.89 ^b
18:3n6	0.34 ± 0.01 ^a	0.27 ± 0.02 ^a	0.14 ± 0.03 ^b
20:2n6	0.21 ± 0.01 ^{ab}	0.25 ± 0.01 ^a	0.15 ± 0.03 ^b
20:3n6	1.58 ± 0.08 ^a	2.06 ± 0.13 ^b	1.96 ± 0.09 ^b
20:4n6	5.40 ± 0.38 ^a	8.89 ± 0.88 ^b	17.70 ± 0.83 ^c
22:2n6	0.04 ± 0.01 ^a	0.028 ± 0.002 ^a	0.39 ± 0.10 ^b
22:4n6	0.08 ± 0.01 ^a	0.10 ± 0.01 ^a	0.59 ± 0.07 ^b
22:5n6	0.058 ± 0.004 ^a	0.09 ± 0.01 ^a	2.19 ± 0.12 ^b
Total n-6 PUFA	30.25 ± 0.58 ^a	32.05 ± 0.80 ^a	39.65 ± 0.68 ^b
18:3n3	0.75 ± 0.05 ^a	0.49 ± 0.05 ^b	0.025 ± 0.002 ^c
20:3n3	0.05 ± 0.01	0.042 ± 0.003	0.17 ± 0.09
20:5n3	1.02 ± 0.04 ^a	0.94 ± 0.07 ^a	0.21 ± 0.06 ^b
22:5n3	0.34 ± 0.02 ^a	0.28 ± 0.02 ^a	0.63 ± 0.13 ^b
22:6n3	6.54 ± 0.14 ^a	6.91 ± 0.19 ^a	0.90 ± 0.07 ^b
Total n-3 PUFA	8.71 ± 0.16 ^a	8.67 ± 0.21 ^a	1.94 ± 0.28 ^b
16:1n7/16:0	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
18:1n9/18:0	1.61 ± 0.10	1.83 ± 0.11	1.50 ± 0.06
20:4n6/20:3n6	3.42 ± 0.16 ^a	4.38 ± 0.38 ^a	9.19 ± 0.51 ^b
20:4n6/20:5n3	5.41 ± 0.46 ^a	10.52 ± 1.51 ^a	207.79 ± 42.69 ^b
20:4n6/22:6n3	0.83 ± 0.06 ^a	1.28 ± 0.12 ^a	21.17 ± 1.80 ^b
20:5n3/22:6n3	0.16 ± 0.01	0.14 ± 0.01	0.24 ± 0.06
22:5n6/22:6n3	0.009 ± 0.001 ^a	0.013 ± 0.002 ^a	2.43 ± 0.29 ^b
22:5n6+22:6n3	6.60 ± 0.14 ^a	7.00 ± 0.20 ^a	3.09 ± 0.15 ^b
n-6/n-3	3.47 ± 0.05 ^a	3.70 ± 0.11 ^a	20.42 ± 5.12 ^b
n-6+n-3	38.96 ± 0.71 ^a	40.72 ± 0.89 ^{ab}	41.59 ± 0.55 ^b
Total fatty acids (µg/ml plasma)	5.48 ± 0.29 ^a	5.50 ± 0.33 ^a	4.28 ± 0.32 ^b

Each parameter is presented as the mean (±SEM). Means with different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$

Table 7

Heart fatty acid composition of the two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

	Dietary group		
	Dam-reared (n = 16)	n-3 Adq (n = 14)	n-3 Def (n = 14)
Fatty acids			
10:0	0.019 ± 0.001	0.019 ± 0.002	0.022 ± 0.002
12:0	0.61 ± 0.04	0.59 ± 0.07	0.61 ± 0.06
14:0	1.72 ± 0.05	1.83 ± 0.06	1.70 ± 0.05
16:0	12.99 ± 0.13 ^a	13.48 ± 0.23 ^a	11.57 ± 0.14 ^b
18:0	17.31 ± 0.21 ^a	16.16 ± 0.37 ^b	16.68 ± 0.24 ^a
20:0	0.35 ± 0.01 ^a	0.30 ± 0.01 ^b	0.29 ± 0.01 ^b
22:0	0.26 ± 0.01	0.26 ± 0.01	0.24 ± 0.01
24:0	0.06 ± 0.02 ^a	0.02 ± 0.01 ^a	0.163 ± 0.004 ^b
Total saturates	33.33 ± 0.28 ^a	32.65 ± 0.22 ^a	31.28 ± 0.25 ^b
12:1	0.020 ± 0.002 ^a	0.018 ± 0.001 ^a	0.029 ± 0.002 ^b
14:1	0.66 ± 0.01 ^a	0.73 ± 0.02 ^b	0.67 ± 0.01 ^a
16:1n7	0.98 ± 0.10	1.13 ± 0.15	1.31 ± 0.11
18:1n7	2.08 ± 0.09 ^a	2.57 ± 0.09 ^b	2.63 ± 0.14 ^b
18:1n9	6.99 ± 0.29 ^a	9.04 ± 1.01 ^{ab}	10.35 ± 0.45 ^b
20:1n9	0.28 ± 0.01 ^{ab}	0.24 ± 0.02 ^a	0.33 ± 0.02 ^b
22:1n9	0.046 ± 0.002	0.045 ± 0.002	0.05 ± 0.01
24:1n9	0.06 ± 0.01 ^a	0.10 ± 0.04 ^a	0.19 ± 0.01 ^b
Total monounsaturates	11.12 ± 0.40 ^a	13.88 ± 1.12 ^b	15.56 ± 0.50 ^b
18:2n6	10.87 ± 0.64 ^a	9.15 ± 0.47 ^a	14.56 ± 0.55 ^b
18:3n6	0.25 ± 0.01	0.22 ± 0.01	0.23 ± 0.01
20:2n6	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
20:3n6	0.93 ± 0.04 ^a	0.99 ± 0.03 ^a	1.13 ± 0.04 ^b
20:4n6	3.73 ± 0.12 ^a	4.35 ± 0.19 ^a	11.63 ± 0.27 ^b
22:2n6	0.017 ± 0.001 ^a	0.021 ± 0.001 ^a	0.14 ± 0.03 ^b
22:4n6	0.12 ± 0.01	0.18 ± 0.01	0.51 ± 0.21
22:5n6	0.15 ± 0.01 ^a	0.22 ± 0.03 ^a	14.28 ± 0.71 ^b
Total n-6 PUFA	16.23 ± 0.57 ^a	15.28 ± 0.47 ^a	42.63 ± 0.58 ^b
18:3n3	0.24 ± 0.01 ^a	0.20 ± 0.02 ^a	0.021 ± 0.001 ^b
20:3n3	0.027 ± 0.001 ^a	0.024 ± 0.001 ^a	0.06 ± 0.01 ^b
20:5n3	0.126 ± 0.004 ^a	0.09 ± 0.01 ^b	0.024 ± 0.003 ^c
22:5n3	0.93 ± 0.03 ^a	0.94 ± 0.04 ^a	0.31 ± 0.02 ^b
22:6n3	32.48 ± 0.59 ^a	31.05 ± 0.89 ^a	2.78 ± 0.10 ^b
Total n-3 PUFA	33.80 ± 0.59 ^a	32.31 ± 0.90 ^a	3.19 ± 0.11 ^b
16:1n7/16:0	0.08 ± 0.01 ^a	0.08 ± 0.01 ^a	0.11 ± 0.01 ^b
18:1n9/18:0	0.41 ± 0.02 ^b	0.58 ± 0.09 ^a	0.63 ± 0.03 ^a
20:4n6/20:3n6	4.11 ± 0.22 ^a	4.48 ± 0.28 ^a	10.43 ± 0.32 ^b
20:4n6/20:5n3	29.96 ± 1.48 ^a	49.56 ± 4.47 ^a	550.68 ± 45.88 ^b
20:4n6/22:6n3	0.12 ± 0.004 ^a	0.14 ± 0.01 ^a	4.25 ± 0.19 ^b
20:5n3/22:6n3	0.004 ± 0.0002 ^a	0.003 ± 0.0002 ^a	0.009 ± 0.001 ^b
22:5n6/22:6n3	0.0045 ± 0.0004 ^a	0.007 ± 0.001 ^a	5.13 ± 0.35 ^b
22:5n6+22:6n3	32.62 ± 0.59 ^a	31.27 ± 0.89 ^a	17.07 ± 0.72 ^b
n-6/n-3	0.48 ± 0.02 ^a	0.48 ± 0.02 ^a	13.60 ± 0.56 ^b
n-6+n-3	50.03 ± 0.46 ^a	47.58 ± 1.02 ^b	45.82 ± 0.56 ^b
Total fatty acids (µg/mg wet heart)	23.53 ± 0.31	24.33 ± 0.71	23.94 ± 0.59

Each parameter is presented as the mean (±SEM). Different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$

Table 8

Lipid class distribution in mouse heart of two artificially reared groups and a dam-reared reference group at 20 week of age

Phospholipids class	Diets		
	Dam-reared (<i>n</i> = 8)	n-3 Adq (<i>n</i> = 7)	n-3 Def (<i>n</i> = 8)
Phosphatidylcholine	43.0 ± 1.0 ^b	43.4 ± 0.9 ^b	39.0 ± 0.9 ^a
Phosphatidylethanolamine	21.8 ± 0.6 ^{ab}	19.9 ± 0.9 ^a	22.8 ± 0.6 ^b
Neutral lipids	20.8 ± 0.6 ^{ab}	19.5 ± 0.8 ^a	22.3 ± 0.6 ^b
Cardiolipin	5.8 ± 0.3 ^b	7.8 ± 0.5 ^a	5.9 ± 0.3 ^b
Phosphatidylserine	1.1 ± 0.2	1.6 ± 0.3	1.9 ± 0.2
Phosphatidylinositol	0.6 ± 0.1 ^a	1.4 ± 0.4 ^{ab}	2.0 ± 0.2 ^b

Values given are mean ± SEM for peak areas of HPLC chromatogram. Different alphabetical superscripts (a, b) indicate significant differences across diets by Tukey's HSD at $P < 0.05$. Neutral lipids included triacylglycerol, cholesterol, and cholesteryl esters

Table 9

The fatty acid composition of phosphatidylethanolamine, phosphatidylcholine and neutral lipids in mouse heart of two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

Fatty acids	Phosphatidylethanolamine			Phosphatidylcholine			Neutral lipids		
	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)
Non-essential									
12:0	0.05 ± 0.01	0.05 ± 0.01	0.031 ± 0.002	0.34 ± 0.22	0.08 ± 0.01	0.08 ± 0.01	5.02 ± 0.82	4.18 ± 0.34	4.78 ± 0.49
14:0	0.30 ± 0.13	0.41 ± 0.17	0.44 ± 0.14	0.60 ± 0.13 ^b	0.42 ± 0.05 ^{ab}	0.29 ± 0.04 ^a	5.79 ± 0.38	4.99 ± 0.30	5.19 ± 0.39
16:0	6.26 ± 0.28 ^b	6.58 ± 0.42 ^b	4.73 ± 0.17 ^a	24.67 ± 3.99	21.65 ± 1.37	17.66 ± 0.34	18.84 ± 0.65	18.67 ± 0.75	17.09 ± 0.91
18:0	26.79 ± 0.47	25.29 ± 0.60	26.34 ± 0.45	27.52 ± 5.02	21.96 ± 0.57	22.24 ± 0.43	5.98 ± 0.48	4.68 ± 0.48	4.68 ± 0.50
20:0	0.28 ± 0.07	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.13 ± 0.03	0.55 ± 0.06 ^b	0.36 ± 0.05 ^{ab}	0.33 ± 0.06 ^a
16:1	0.32 ± 0.02	0.28 ± 0.01	0.30 ± 0.04	0.51 ± 0.08	0.40 ± 0.07	0.48 ± 0.05	5.75 ± 0.75	6.13 ± 1.11	7.07 ± 1.16
18:1n9	3.53 ± 0.15 ^a	4.37 ± 0.13 ^b	4.94 ± 0.21 ^b	7.28 ± 1.40 ^b	6.01 ± 0.16 ^b	11.12 ± 0.55 ^a	23.25 ± 1.15 ^a	28.73 ± 0.69 ^b	29.05 ± 1.06 ^b
18:1n7	0.74 ± 0.06 ^a	1.07 ± 0.09 ^b	1.40 ± 0.10 ^c	2.30 ± 0.44	2.37 ± 0.15	3.20 ± 0.12	2.51 ± 0.21 ^b	3.39 ± 0.33 ^{ab}	3.59 ± 0.34 ^a
20:1n9	0.21 ± 0.02 ^b	0.17 ± 0.02 ^b	0.31 ± 0.04 ^a	0.24 ± 0.08	0.16 ± 0.01	0.22 ± 0.05	1.23 ± 0.12 ^{ab}	0.84 ± 0.06 ^b	1.36 ± 0.16 ^a
n-6 PUFA									
18:2n6	2.01 ± 0.10 ^a	1.31 ± 0.08 ^b	2.51 ± 0.15 ^c	6.92 ± 0.75 ^b	4.39 ± 0.36 ^a	7.78 ± 0.57 ^b	16.41 ± 0.91 ^b	13.76 ± 0.53 ^a	11.46 ± 0.29 ^a
20:2n6	0.07 ± 0.02 ^b	0.046 ± 0.004 ^b	0.16 ± 0.04 ^a	0.12 ± 0.02	0.14 ± 0.04	0.15 ± 0.04	0.24 ± 0.03	0.36 ± 0.16	0.56 ± 0.18
20:3n6	0.20 ± 0.01 ^b	0.23 ± 0.01 ^b	0.33 ± 0.01 ^a	0.57 ± 0.13 ^b	0.50 ± 0.02 ^b	1.01 ± 0.05 ^a	0.46 ± 0.05	0.51 ± 0.09	0.60 ± 0.11
20:4n6	3.59 ± 0.22 ^b	4.23 ± 0.23 ^b	13.29 ± 0.22 ^a	2.61 ± 0.48 ^b	3.10 ± 0.38 ^b	11.81 ± 0.38 ^a	1.22 ± 0.12 ^b	1.50 ± 0.27 ^b	3.03 ± 0.40 ^a
22:4n6	0.12 ± 0.02 ^b	0.21 ± 0.02 ^b	2.34 ± 0.12 ^a	0.28 ± 0.12 ^b	0.15 ± 0.05 ^b	1.88 ± 0.09 ^a	0.11 ± 0.02 ^b	0.18 ± 0.03 ^b	0.64 ± 0.13 ^a
22:5n6	0.47 ± 0.08 ^b	0.54 ± 0.09 ^b	23.17 ± 1.17 ^a	0.72 ± 0.21 ^b	0.50 ± 0.12 ^b	12.16 ± 0.85 ^a	0.21 ± 0.08 ^b	0.22 ± 0.07 ^b	1.25 ± 0.22 ^a
n-3 PUFA									
18:3n3	0.34 ± 0.06	0.20 ± 0.03	0.21 ± 0.05	0.077 ± 0.004 ^a	0.05 ± 0.01 ^b	0.015 ± 0.005 ^c	1.02 ± 0.06 ^a	0.84 ± 0.03 ^b	0.11 ± 0.02 ^c
20:3n3	0.14 ± 0.01 ^a	0.09 ± 0.01 ^b	0.014 ± 0.003 ^c	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.16 ± 0.01 ^b	0.12 ± 0.02 ^b	0.020 ± 0.005 ^a
22:3n3	0.82 ± 0.05 ^b	0.91 ± 0.06 ^b	0.405 ± 0.025 ^a	1.15 ± 0.23 ^b	1.06 ± 0.06 ^b	0.30 ± 0.04 ^a	0.57 ± 0.06 ^b	0.59 ± 0.12 ^b	0.044 ± 0.004 ^a
22:6n3	40.64 ± 0.74 ^b	39.59 ± 1.05 ^b	4.56 ± 0.19 ^a	27.92 ± 0.72 ^b	29.21 ± 0.65 ^b	1.93 ± 0.08 ^a	3.72 ± 0.45 ^b	3.48 ± 0.55 ^b	0.31 ± 0.12 ^a
22:5n6/22:6n3	0.012 ± 0.002 ^b	0.014 ± 0.003 ^b	5.17 ± 0.39 ^a	0.021 ± 0.004 ^b	0.018 ± 0.005 ^b	6.26 ± 0.31 ^a	0.05 ± 0.02 ^b	0.06 ± 0.02 ^b	5.55 ± 0.96 ^a
22:5n6+22:6n3	41.11 ± 0.68 ^b	40.13 ± 0.97 ^b	27.73 ± 1.11 ^a	33.94 ± 5.50 ^b	29.71 ± 0.57 ^b	14.09 ± 0.91 ^a	3.93 ± 0.50 ^b	3.66 ± 0.57 ^b	1.56 ± 0.32 ^a
n-6+n-3	48.39 ± 0.78	47.34 ± 0.89	47.00 ± 0.50	45.65 ± 7.08	39.10 ± 0.82	37.02 ± 0.84	24.12 ± 1.42 ^b	21.53 ± 1.55 ^{ab}	18.07 ± 1.01 ^a
Total fatty acids (µg/mg)	0.39 ± 0.04	0.48 ± 0.07	0.39 ± 0.06	0.27 ± 0.03	0.32 ± 0.06	0.31 ± 0.03	0.45 ± 0.06	0.52 ± 0.11	0.44 ± 0.07

Each parameter is presented as the mean (±SEM). Different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at *P* < 0.05. Neutral lipids included triacylglycerol, cholesterol, and cholesteryl esters

Table 10

The fatty acid composition of phosphatidylserine, phosphatidylinositol and cardiolipin in mouse heart of two artificially reared groups and a dam-reared reference group at 20 week of age (wt % of total fatty acids)

Fatty acids	Phosphatidylserine			Phosphatidylinositol			Cardiolipin		
	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)	Dam-reared (n = 8)	n-3 Adq (n = 7)	n-3 Def (n = 8)
Non-essential									
16:0	20.34 ± 2.18	17.38 ± 1.75	15.04 ± 1.18	25.10 ± 1.97	21.75 ± 1.21	19.97 ± 1.31	2.97 ± 0.41	2.95 ± 0.41	2.50 ± 0.45
18:0	41.18 ± 1.92	42.17 ± 1.46	39.99 ± 1.80	38.34 ± 1.49 ^{ab}	39.62 ± 0.85 ^b	32.75 ± 2.37 ^a	3.47 ± 0.64	3.21 ± 0.65	3.82 ± 0.75
16:1	3.11 ± 0.19	2.88 ± 0.34	2.64 ± 0.17	3.59 ± 0.36	3.28 ± 0.50	2.55 ± 0.20	2.17 ± 0.13	2.03 ± 0.22	2.11 ± 0.12
18:1n9	4.72 ± 0.40	5.89 ± 0.43	5.13 ± 0.37	4.96 ± 0.42	6.02 ± 0.52	6.17 ± 0.38	9.81 ± 0.74 ^b	14.84 ± 0.70 ^a	8.59 ± 0.87 ^b
18:1n7	0.81 ± 0.17	1.27 ± 0.19	1.11 ± 0.16	0.74 ± 0.09 ^b	1.16 ± 0.13 ^{ab}	1.57 ± 0.15 ^a	4.56 ± 0.37 ^b	8.22 ± 0.57 ^a	3.57 ± 0.26 ^b
n-6 PUFA									
18:2n6	5.34 ± 0.87 ^b	4.48 ± 0.79 ^b	12.14 ± 2.23 ^a	2.53 ± 0.37 ^b	2.71 ± 0.23 ^b	9.47 ± 2.81 ^a	54.55 ± 2.20 ^{ab}	40.79 ± 1.77 ^b	63.40 ± 3.11 ^c
20:3n6	0.69 ± 0.08	0.95 ± 0.11	0.89 ± 0.07	0.34 ± 0.04 ^b	0.44 ± 0.09 ^{ab}	0.91 ± 0.22 ^a	2.64 ± 0.23 ^b	3.46 ± 0.17 ^a	2.92 ± 0.23 ^{ab}
20:4n6	1.82 ± 0.45 ^b	1.76 ± 0.17 ^b	3.38 ± 0.28 ^a	1.81 ± 0.22 ^b	2.40 ± 0.35 ^b	5.12 ± 0.65 ^a	0.71 ± 0.06 ^b	0.95 ± 0.05 ^b	1.46 ± 0.11 ^a
22:4n6	0.28 ± 0.03 ^b	0.32 ± 0.05 ^b	0.87 ± 0.15 ^a	0.33 ± 0.07	0.30 ± 0.07	0.55 ± 0.09	0.03 ± 0.01 ^b	0.02 ± 0.005 ^b	0.35 ± 0.09 ^a
22:5n6	1.28 ± 0.16 ^b	1.32 ± 0.21 ^b	7.08 ± 0.98 ^a	1.55 ± 0.13 ^b	1.50 ± 0.29 ^b	7.07 ± 0.77 ^a	0.39 ± 0.09 ^b	0.41 ± 0.08 ^b	3.13 ± 0.36 ^a
n-3 PUFA									
22:5n3	0.29 ± 0.03 ^b	0.30 ± 0.04 ^b	0.009 ± 0.002 ^a	ND	ND	ND	0.15 ± 0.02 ^a	0.23 ± 0.02 ^b	0.08 ± 0.01 ^a
22:6n3	5.85 ± 0.80 ^b	7.60 ± 1.05 ^b	0.99 ± 0.12 ^a	4.95 ± 0.83 ^b	6.82 ± 1.32 ^b	0.85 ± 0.11 ^a	9.79 ± 0.87 ^a	14.57 ± 0.88 ^b	0.37 ± 0.04 ^c
22:5n6/22:6n3	0.27 ± 0.08 ^b	0.22 ± 0.07 ^b	7.48 ± 1.18 ^a	0.38 ± 0.06 ^b	0.31 ± 0.11 ^b	8.64 ± 0.55 ^a	0.04 ± 0.01 ^b	0.03 ± 0.01 ^b	8.46 ± 0.66 ^a
22:5n6+22:6n3	7.13 ± 0.75	8.92 ± 1.00	8.07 ± 1.05	6.50 ± 0.79	8.32 ± 1.17	7.92 ± 0.88	10.18 ± 0.86 ^a	14.98 ± 0.83 ^b	3.50 ± 0.39 ^c
n-6+n-3	15.55 ± 1.07 ^b	16.73 ± 1.55 ^b	25.36 ± 2.77 ^a	11.51 ± 1.06 ^b	14.18 ± 1.69 ^b	23.97 ± 3.34 ^a	68.26 ± 1.70 ^{ab}	60.44 ± 2.07 ^b	71.70 ± 2.58 ^a
Total fatty acids (ng/mg)	7.55 ± 0.32	7.87 ± 1.25	7.95 ± 1.47	7.02 ± 0.85	9.22 ± 2.49	9.40 ± 1.17	63.80 ± 10.98	96.83 ± 33.53	78.66 ± 18.17

Each parameter is presented as the mean (±SEM). Different alphabetical superscripts (a, b, c) indicate significant differences across diets by Tukey's HSD at $P < 0.05$ ND not detected (i.e., <0.01%)