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# Age-Related Changes in Mitochondrial Membrane Composition of *Nothobranchius rachovii*

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Mitochondrial membrane composition may be a critical factor in the mechanisms of the aging process by influencing the propagation of reactions involved in mitochondrial function during periods of high stress. Changes affecting either lipid class or fatty acid compositions could affect phospholipid properties and alter mitochondrial function and cell viability. In the present study, mitochondrial membrane phospholipid compositions were analyzed throughout the life cycle of *Nothobranchius rachovii*. Mitochondrial phospholipids showed several changes with age. Proportions of cardiolipin decreased and those of sphingomyelin increased between 11- and 14-month-old fish. Fatty acid compositions of individual phospholipids in mitochondria were also significantly affected with age. These data suggest increasing damage to mitochondrial lipids during the life cycle of *N. rachovii* that could be one of the main factors related with and contributing to degraded mitochondrial function associated with the aging process.

**Key Words:** Aging—Mitochondria—Phospholipid—Fatty acid—Fish.

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AGING is a complex biological process affecting all organisms and consisting of progressive cumulative impairment of cellular bioenergetics function, increased oxidative stress, and an augmented risk of age-associated disorders affecting many tissues (1). Mitochondria, the powerhouses of the cell, have been implicated as key organelles contributing to the timing and severity of deterioration associated with the aging process (2,3). According to the mitochondrial theory of aging, reactive oxygen species, produced by the respiratory electron transport chain under normal physiological conditions, cause damage to the organelle proteins, lipids, and DNA leading to mitochondrial dysfunction and, thus, to cell and tissue decay (1,4,5). Although the precise cause–effect relationship among the alterations with age observed inside mitochondria remains unclear, it could be suggested that mitochondrial membrane lipids may be pacemakers of such events, determining how the changes propagate. Thus, membrane lipid composition has been shown to be linked to metabolic rate and life span in a wide number of animal species (6). In comparative studies, performed on various species of mammals, birds, and reptiles, it has been found that species with a shorter life span have more unsaturated membranes (plasma and mitochondrial) than species with longer life span (7). Membranes with high levels of polyunsaturated fatty acids (PUFA) are more fluid and this can enable or promote higher molecular activity of membrane proteins and, in turn, increase the metabolic activity of cells, tissues and, consequently, whole animals. At the same time, susceptibility to oxidative damage increases with the proportion of PUFA in membranes (8). Lipid peroxidation constitutes the major oxidative process inside cells, resulting in the production of multiple highly reactive derivatives that cause further damage to other membrane components, proteins, and nucleic acids (3). These oxidized lipid derivatives also induce adaptive responses that act to decrease oxidative damage and enhance antioxidant defenses (9). Therefore, membrane lipids (mainly those from mitochondria) would be one of the main regulators in the processes associated with oxidative damage accumulation and aging.

It is known that mitochondrial membranes in mammals have a distinctive composition of lipid classes, phospholipids (PL), glycolipids, and cholesterols (10) related to the role of mitochondria in energy metabolism and oxygen consumption (11). Mitochondrial inner membrane is constituted of 80% proteins (mainly electron transport chain components) and 20% lipids, which contain a high percentage of the PL, cardiolipin (CL), and essentially no cholesterol (12). CLs play a pivotal role in the organelle functions and are almost exclusively located in the vicinity of the mitochondrial electron transport chain components and, thus, close to the site of reactive oxygen species production. Moreover, CLs have a unique fatty acid composition with high levels of PUFA, which makes them particularly prone to peroxidative damage (13). A deterioration of CL content and/or composition could lead to mitochondrial

dysfunction and cell decay. Mitochondrial membranes also contain small amounts of sphingomyelin (SM), which has membrane-rigidifying properties (due to its low content in PUFA) and may retard the lateral propagation of free radicals (14,15). Other PL species such as phosphatidylserine (PS) and phosphatidylinositol (PI) are also important as they are precursors for signaling molecules, some associated with apoptosis, the ultimate process taking place in cells with dysfunctional mitochondria (16–18).

The overall aim of the present study was to characterize changes in mitochondrial membrane PLs with age over the whole life cycle of the annual killifish, *Nothobranchius rachovii*. *Nothobranchius* are a fish genus characterized by having an extremely short life span (3–18 months, depending on the species) and, as such, species within this group have been well studied and show a progressive deterioration in several aging markers (19–22). In this study, we focused on alterations to PL composition and fatty acid compositions of specific individual PL that may be critical in the modulation of mitochondrial function during phases of high oxidative stress, such as aging, that result in molecular damage.

## Methods

### *Animal Housing and Sampling*

To perform this study, a population ($n$ = 42) of *N. rachovii* (Actinopterygii, Cyprinodontiformes, and Nothobranchiidae) with the same genetic origin (Beira population, Beira 98, Mozambique) was reared and maintained throughout their whole life cycle on the same rearing and feeding conditions in the Fish Chronobiology facilities of the University of Murcia. Fish (males and females) were kept under constant temperature (26 ± 2°C) in 70-L tanks equipped with a recirculating fresh water system (4 L/h flow) and biological and mechanical filtration. The photoperiod was set at 12L:12D (200 lux of light intensity at the water surface). Fish were fed frozen bloodworms (*Chiromonus*, Frozen Fishfood, Holland) twice per day. The fatty acid composition of the diet is presented in Table 1. The number of dead fish was recorded daily to obtain the survival curve of the population by the Kaplan–Meier method.

In order to obtain isolated mitochondria from *N. rachovii* at different ages, fish of 3, 7, 11, and 14 months were euthanized by exposure to the anesthetic MS222 (200 mg/L), weight measured, and whole animals homogenized in a blender to produce a pate that served as a source of material for analyses. These specimens were not considered to elaborate the survival curve of the population. Four replicate samples of each age group were collected for lipid and fatty acid analysis. In order to obtain sufficient material for all the required analyses, 3- and 7-month-old fish samples were pools of two whole bodies, whereas sample replicates of older fish were individuals. The experimental procedure conformed to current Spanish law on animal experiments, and the experimental protocol was approved by the Bioethics Committee of the University of Murcia.

Table 1. Fatty Acid Composition (percentage of total fatty acids) of *Nothobranchius rachovii* Diet

| Fatty Acid | Frozen Bloodworm |
|---|---|
| 14:0 | 3.8 |
| 16:0 | 20.4 |
| 18:0 | 5.8 |
| ΣSaturated | 31.7 |
| 16:1n-7 | 18.9 |
| 18:1n-9 | 7.7 |
| 18:1n-7 | 6.8 |
| ΣMonounsaturated | 34.0 |
| 18:2n-6 | 11.2 |
| 20:4n-6 | 1.6 |
| Σn-6 PUFA | 14.1 |
| 18:3n-3 | 4.8 |
| 20:5n-3 | 13.3 |
| Σn-3 PUFA | 18.8 |
| ΣPUFA | 33.0 |

*Notes*: PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

### *Mitochondria Isolation*

Approximately 1 g of fresh (nonfrozen) whole fish pate was homogenized in 8-mL ice-cold sucrose buffer (0.4 M phosphate buffer pH 7.4, 0.25 M sucrose, 0.15 M KCl, 40 mM KF, and 1 mM *N*-acetyl-cysteine) using a tissue disrupter (IKA T25 digital Ultra-Turrax Fisher Scientific, Loughborough, UK). Sucrose buffer homogenates were then centrifuged at 600*g* twice for 6 minutes and the pellet discarded (cell/nuclei debris). Supernatants were then centrifuged at 6,800*g* twice for 10 minutes and the resulting pellet was used for lipid extraction. To verify that pellets were highly enriched in mitochondria, a portion of our isolates was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4°C and then processed prior to analysis by transmission electron microscopy (Tecnai G2 Spirit BioTWIN, FEI Europe, Eindhoven, The Netherlands) as described in a previous work (23). Purity of preparations was also tested by measuring total superoxide dismutase and superoxide dismutase 2 (mitochondria specific) in our mitochondria isolates (as described in Ref. (24)). Superoxide dismutase 2 activity in mitochondria pellets always represented more than 95% of total superoxide dismutase activity.

### *Lipid Extraction and PL class Composition*

Total lipid from whole-animal (males and females) mitochondria was obtained by extraction with chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene as antioxidant, basically according to Folch and colleagues (25). Briefly, mitochondrial pellets were

homogenized in 5 mL of ice-cold chloroform/methanol followed by the addition of 1 mL of 0.88% (w/v) KCl, mixing, and layers allowed to separate on ice for 1 h. The upper aqueous layer was aspirated and the lower organic layer was evaporated under a stream of oxygen-free nitrogen. All lipids extracts were stored at −20°C under a $N_2$ atmosphere prior to analysis.

PL classes were separated by high-performance thin-layer chromatography using 10- × 10-cm silica gel plates (VWR, Lutterworth, England) and methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9, by volume) as solvent system (26). The lipid classes were visualized by charring at 160°C for 15 minutes after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 thin layer chromatography (TLC) scanner (version Firmware 1.14.16) (27). Scanned images were recorded automatically and analyzed by computer using winCATS (Planar Chromatography Manager, version 1.2.0).

### *PL Fatty Acid Composition*

Individual PL classes from whole-animal mitochondria were separated by preparative TLC, using silica gel plates (20 × 20 cm; VWR) and the solvent system as above. Individual PL classes were identified by comparison with known standards after spraying with 1% (w/v) 2′,7′-dichlorofluorescein in 97% (v/v) methanol containing 0.05% (w/v) butylated hydroxytoluene and visualization under UV light (UVGL-58 Minerallight Lamp, Ultraviolet Prod. Inc., San Gabriel, CA). Each PL class was scraped from the plate into a test tube and subjected directly (on silica) to acid-catalyzed transmethylation at 50°C overnight following addition of 2 mL of 1% (v/v) sulfuric acid in methanol in order to obtain the fatty acid methyl esters (28). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m × 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, UK) and on-column injection at 50°C. Hydrogen was used as a carrier gas and temperature programming was from 50 to 150°C at 40°C/min and then to 230°C at 2.0°C/min. Individual methyl esters were identified by comparison with known standards. Data were collected and processed using Chromcard for Windows (version 1.19).

### *Indexes and Statistical Analysis*

The long-chain polyunsaturated fatty acids index corresponds to the sum of long-chain polyunsaturated fatty acids, fatty acids with 20 or more carbons and 2 or more double bounds. The peroxidation index (PIn) was used as an estimate of PL susceptibility to oxidation and was calculated using the formula: PIn = 0.025 × (percentage of monoenoics) + 1 × (percentage of dienoics) + 2 × (percentage of trienoics) + 4 × (percentage of tetraenoics) + 6 × (percentage of pentaenoics) + 8 × (percentage of hexaenoics) (29). Results are presented as mean ± *SD* ($n = 4$). Data were checked for homogeneity of variances by the Levene's test and, where necessary, arc-sin transformed before further statistical analysis. One-way analysis of variance was performed to determine statistical significance of differences between age groups for fish weight, each PL class, single fatty acid, group of fatty acids, or index, and Tukey's post hoc test was used for multiple comparisons when pertinent. $p < .05$ was considered to be statistically different. A Pearson correlation test was performed for each PL class, single fatty acid, group of fatty acids, or index with age. Two levels of statistical significance of differences, $p < .05$ and $p < .01$, were considered. All data were processed using SPSS Statistical Software System version 15.0 (SPSS Inc., Chicago, IL).

## Results

### Nothobranchius rachovii *Life Span and Aging*

Kaplan–Meier analysis gave a mean life span of 13.5 months and a maximum survival of 15 months (Figure 1). Fish doubled their weight between 3 and 11 months (from 0.4 to 0.8 g), whereas no growth between 11- and 14-month-old animals was registered (Figure 2). During this last aging period, fish suffered changes in their morphology as spine curvature, body coloration loss, and progressive deterioration of the fins, especially caudal fin.

### *PL class Composition of Whole-Fish Mitochondria*

Data from male and female fish were combined in the present study as they did not differ in their lipid profiles. The PL class compositions of whole-fish mitochondria from 3-, 7-, 11-, and 14-month-old *N. rachovii* are shown in Figure 3. Phosphatidylcholine (PC) was the predominant PL class, representing 40.7% of total PL in 3-month-old animals. The next PL in abundance was phosphatidylethanolamine (PE), which constituted 33.9% of the total PL. Thus, the sum of both PC and PE represented nearly 75% of total PL in 3-month-old fish. The remaining 25% of PL was, in rank order, PS, SM, and PI representing 8.6%, 7.7%, and 6.4% of total PL, respectively. Perhaps surprisingly, CL was the least abundant PL class in mitochondria of 3-month-old *N. rachovii* constituting only 2.7% of total PL.

Some changes with age were observed in *N. rachovii* mitochondrial PL class composition (Figure 3). Regarding the two major PL classes, there was a progressive decrease in PC content from 3- to 14-month-old animals (from 40.7% to 30.9%), as reflected by the Pearson correlation test ($r = -.561$), whereas no significant changes in the proportions of PE were found among the different age groups. Consequently, the sum of PC and PE in 14-month-old *N. rachovii* was significantly lower than in younger individuals (65% vs 74.6%). Proportion of CL significantly

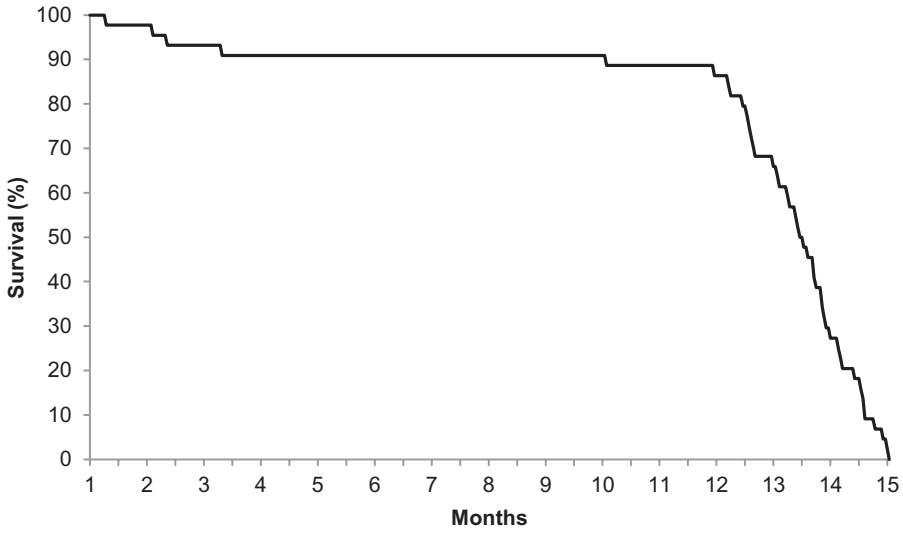


Figure 1. Survival rate of *Nothobranchius rachovii* from 1 month old ($n = 42$).

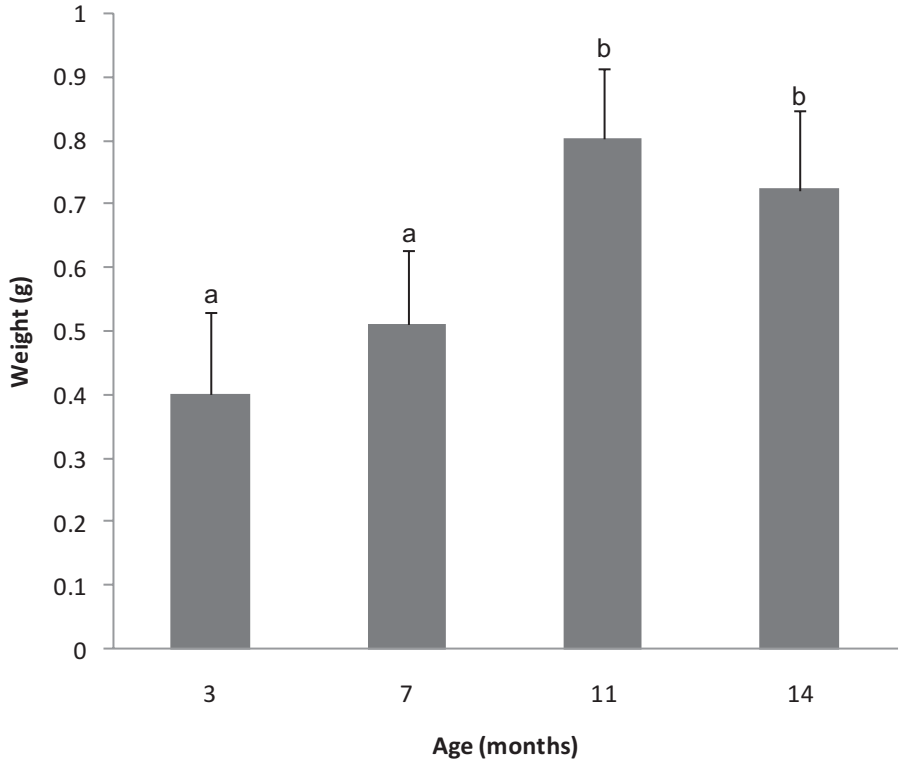


Figure 2. Average weight (mean ± *SD*) of 3-, 7-, 11-, and 14-month-old *Nothobranchius rachovii* ($n = 10$). Data for total population (males and females) are presented. Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$).

increased between 3- and 11-month-old animals (from 2.7% to 6.0%) but was lower again in 14-month-old fish (3.9%). The decrease in PC and CL contents with age was in part compensated by significant increased proportions of PS from 3- to 7-month-old fish (8.6% vs 11.2%) and, especially, SM from 7- to 14-month-old animals (from 5.0% to 15.6%; Figure 3). In relation to PI, although there were no significant differences between the four age groups, Pearson correlation test denoted a significative trend toward a decrease with age ($r = -.579$).

### *Fatty Acid Compositions of Individual PL of Whole-Fish Mitochondria*

Fatty acid compositions of individual PL classes from whole-body mitochondria of 3-, 7-, 11-, and 14-month-old *N. rachovii* are presented in Tables 2–7. Each PL class had a

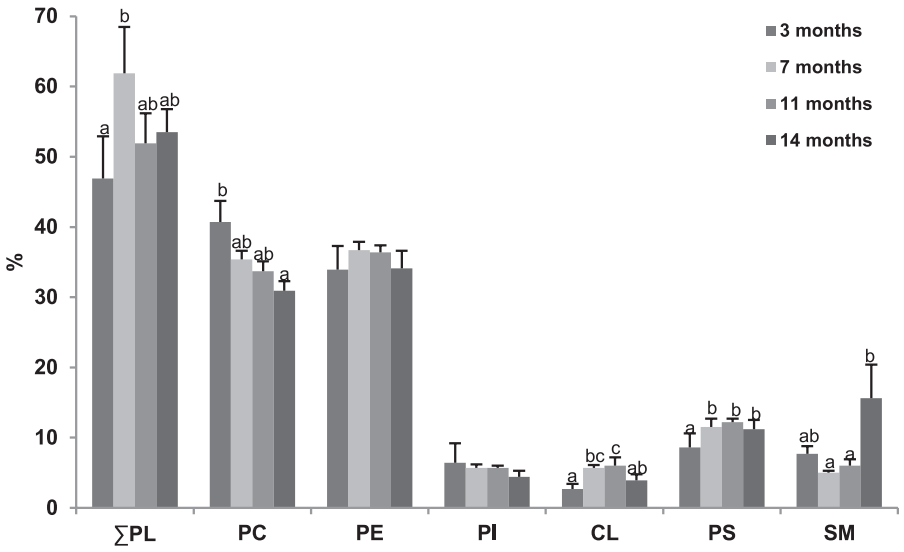


Figure 3. Phospholipid (PL) content (percentage of weight of total lipid) and PL class composition (percentage of total PL) of 3-, 7-, 11-, and 14-month-old *Nothobranchius rachovii*. Results are means ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups for each PL class as determined by one-way analysis of variance ($p < .05$). Pearson correlation values between age and PL class (*$p < .05$)—ΣPL: 0.281, PC: −0.561*, PE: 0.218, PI: −0.579*, CL: 0.263, PS: 0.170, and SM: 0.327. CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; ΣPL, total phospholipids; PS, phosphatidylserine; SM, sphingomyelin.

characteristic fatty acid composition. PC was characterized by high levels of palmitic acid (16:0), oleic acid (18:1n-9), and docosahexaenoic acid (DHA, 22:6n-3; Table 2). Both PE and PS showed high levels of stearic acid (18:0) and DHA, and PE was also characterized by containing dimethyl acetals, produced from the ether PL, plasmalogen (Tables 3 and 4). SM had a highly saturated profile with high proportions of 16:0 and 18:0 and the monounsaturated fatty acid, nervonic acid (24:1n-9; Table 5). PI was characterized by high levels of 18:0 and arachidonic acid (20:4n-6; Table 6), and CL by high levels of linoleic acid (18:2n-6) and n-7 monounsaturated fatty acid (Table 7). The PIn was also different among individual PL from 3-month-old fish, with PE and PS having higher PIn values, whereas SM was the PL class least prone to oxidation.

The fatty acid compositions of individual PL from whole-fish mitochondria showed several significant changes with age. There was a general increase in total saturated fatty acid content in all PL classes, manifested by a positive Pearson correlation coefficient in all the lipid classes, specially PC, PE, SM, and PI. This increase occurred mainly between 11- and 14-month-old animals in PE, PS, and CL (Tables 3, 4, and 7). Proportions of monounsaturated fatty acid increased significantly in PC, PE and, especially, in PS, mainly due to increased oleic acid content (Tables 2–4). In contrast, total n-6 PUFA decreased significantly in PC, PI, SM, and, particularly, CL in which linoleic acid markedly decreased between 11- and 14-month-old animals (20.5% vs 10.5%; Tables 2 and 5–7). Similarly, n-3 PUFA proportions decreased significantly in the two major PL classes, PC and PE, and, especially, PS, mainly due to a marked decrease in DHA between 11- and 14-month-old fish (27.0% vs 16.3%). A general decrease in the percentage of 22:5n-3 correlated with age was also found in all the PL classes. The PIn decreased progressively and significantly in PC and PE and also in PS in which the major decrease occurred between 11- and 14-month-old animals. In SM, PIn increased significantly between 11- and 14-month-old fish.

## Discussion

*Nothobranchius rachovii* showed an average life span of 13.5 months under our laboratory conditions in the present study, differing from the 8.5 months previously described by Herrera and Jagadeeswaran (30). This difference could be attributed to intraspecific variations or to disparities in husbandry conditions such as feeding ration and diet composition, which are parameters currently being actively studied due to their obvious possible implications on animal metabolic rate, oxidative stress, disease, and aging (8,31). During the aging process, fish showed similar morphological changes to those described for *Nothobranchius korthausae* (spine curvature, body coloration loss, and progressive deterioration of the fins, especially caudal fin) (32).

Mitochondria isolated from whole *N. rachovii* showed a distinctive PL composition in which PC and PE predominated, representing more than 60% of total PL, PS, PI, and SM had similar proportions and CL just constituted less than 3% of total PL in 3-month-old fish. These were interesting data considering the critical role that CL plays in the mitochondrial inner membrane as a regulator of processes related to oxidative phosphorylation and mitochondrial integrity (13). The proportions of mitochondrial PL showed several changes with age with

Table 2. Fatty Acid Composition (percentage of total fatty acids) of Phosphatidylcholine of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Mo | 7 Mo | 11 Mo | 14 Mo | *r* |
|---|---|---|---|---|---|
| 16:0 | 26.5 ± 0.4 | 26.6 ± 0.3 | 27.1 ± 1.4 | 28.0 ± 0.6 | .621* |
| 18:0 | 7.5 ± 0.1$^{a}$ | 8.5 ± 0.4$^{b}$ | 9.7 ± 0.2$^{c}$ | 9.7 ± 0.5$^{c}$ | .910** |
| ∑Saturated | 36.5 ± 0.3$^{a}$ | 37.6 ± 0.3$^{ab}$ | 39.1 ± 1.5$^{bc}$ | 40.1 ± 0.4$^{c}$ | .883** |
| 16:1n-7 | 4.7 ± 0.7 | 5.5 ± 0.5 | 5.0 ± 0.8 | 4.1 ± 2.4 | .082 |
| 18:1n-9 | 13.5 ± 0.1$^{a}$ | 20.1 ± 0.8$^{b}$ | 20.3 ± 0.6$^{b}$ | 22.0 ± 2.4$^{b}$ | .833** |
| 18:1n-7 | 6.8 ± 0.1$^{c}$ | 3.4 ± 0.3$^{a}$ | 4.2 ± 0.3$^{b}$ | 4.2 ± 0.3$^{b}$ | −.602* |
| ∑Monounsaturated | 26.9 ± 0.2$^{a}$ | 30.8 ± 1.4$^{b}$ | 31.3 ± 0.8$^{b}$ | 32.8 ± 2.6$^{b}$ | .801** |
| 18:2n-6 | 6.2 ± 0.3$^{b}$ | 3.7 ± 0.2$^{a}$ | 4.0 ± 0.1$^{a}$ | 3.7 ± 0.9$^{a}$ | −.713** |
| 20:4n-6 | 1.7 ± 0.1$^{a}$ | 3.4 ± 0.2$^{c}$ | 2.5 ± 0.2$^{b}$ | 2.2 ± 0.3$^{b}$ | .092 |
| 22:4n-6 | 1.0 ± 0.1 | 1.4 ± 0.1 | 1.0 ± 0.2 | 0.9 ± 0.1 | −.297 |
| 22:5n-6 | 1.1 ± 0.2$^{a}$ | 2.0 ± 0.1$^{c}$ | 1.4 ± 0.1$^{c}$ | 1.6 ± 0.2$^{b}$ | .253 |
| ∑n-6 PUFA | 11.2 ± 0.3$^{bc}$ | 11.6 ± 0.4$^{c}$ | 9.8 ± 0.5$^{ab}$ | 9.2 ± 1.4$^{a}$ | −.733** |
| 20:5n-3 | 1.7 ± 0.2$^{b}$ | 0.7 ± 0.1$^{a}$ | 0.9 ± 0.2$^{a}$ | 0.8 ± 0.1$^{a}$ | −.462 |
| 22:5n-3 | 6.3 ± 0.3$^{c}$ | 2.4 ± 0.7$^{b}$ | 2.7 ± 0.2$^{b}$ | 1.9 ± 0.3$^{a}$ | −.823** |
| 22:6n-3 | 15.5 ± 0.4$^{b}$ | 14.8 ± 0.7$^{ab}$ | 14.5 ± 1.0$^{ab}$ | 13.5 ± 1.0$^{a}$ | −.679** |
| ∑n-3 PUFA | 23.6 ± 0.5$^{b}$ | 18.1 ± 1.1$^{a}$ | 18.4 ± 1.4$^{a}$ | 16.5 ± 1.2$^{a}$ | −.847** |
| ∑PUFA | 36.6 ± 0.1$^{c}$ | 31.6 ± 1.2$^{b}$ | 29.6 ± 1.2$^{ab}$ | 27.1 ± 2.3$^{a}$ | −.923** |
| ∑n-3 LC-PUFA | 23.6 ± 0.5$^{b}$ | 18.1 ± 1.0$^{a}$ | 18.1 ± 1.3$^{a}$ | 16.5 ± 1.2$^{a}$ | −.836** |
| n-3/n-6 | 2.2 ± 0.1$^{c}$ | 1.6 ± 0.1$^{a}$ | 1.9 ± 0.2$^{bc}$ | 1.8 ± 0.2$^{ab}$ | −.303 |
| PIn | 201.9 ± 2.3$^{c}$ | 179.4 ± 7.7$^{b}$ | 169.6 ± 9.7$^{ab}$ | 156.1 ± 9.9$^{a}$ | −.910** |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

Table 3. Fatty Acid Composition (percentage of total fatty acids) of Phosphatidylethanolamine of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Mo | 7 Mo | 11 Mo | 14 Mo | *r* |
|---|---|---|---|---|---|
| 16:0 DMA | 5.5 ± 0.1 | 4.4 ± 0.6 | 4.6 ± 0.4 | 6.6 ± 2.0 | .306 |
| 16:0 | 8.1 ± 0.9 | 8.0 ± 0.2 | 8.9 ± 0.3 | 8.7 ± 0.8 | .434 |
| 18:0 DMA | 4.0 ± 0.5$^{a}$ | 4.7 ± 0.4$^{ab}$ | 5.3 ± 0.2$^{b}$ | 7.2 ± 0.7$^{c}$ | .906** |
| 18:0 | 15.1 ± 0.3$^{ab}$ | 16.3 ± 0.7$^{b}$ | 15.2 ± 0.5$^{ab}$ | 13.9 ± 1.2$^{a}$ | −.500* |
| ∑Saturated | 34.1 ± 1.6$^{a}$ | 34.6 ± 0.8$^{a}$ | 35.8 ± 0.4$^{a}$ | 37.9 ± 0.9$^{b}$ | .823** |
| 16:1n-7 | 2.3 ± 0.2$^{a}$ | 3.6 ± 0.3$^{b}$ | 4.2 ± 0.3$^{bc}$ | 4.9 ± 0.5$^{c}$ | .932** |
| 18:1n-9 | 4.6 ± 1.0$^{a}$ | 6.0 ± 0.5$^{ab}$ | 6.4 ± 1.9$^{ab}$ | 7.1 ± 0.7$^{c}$ | .654** |
| 18:1n-7 | 5.0 ± 0.3 | 3.4 ± 0.2 | 3.4 ± 2.3 | 4.1 ± 0.6 | −.255 |
| ∑Monounsaturated | 13.2 ± 0.7$^{a}$ | 14.7 ± 0.9$^{ab}$ | 15.9 ± 0.8$^{b}$ | 18.5 ± 1.5$^{c}$ | .897** |
| 18:2n-6 | 3.4 ± 0.6$^{ab}$ | 3.4 ± 0.5$^{ab}$ | 2.7 ± 0.3$^{a}$ | 3.8 ± 0.4$^{b}$ | .132 |
| 20:4n-6 | 4.5 ± 0.3$^{a}$ | 7.0 ± 0.4$^{c}$ | 5.7 ± 0.1$^{b}$ | 5.3 ± 0.2$^{b}$ | .124 |
| 22:4n-6 | 1.7 ± 0.1 | 2.4 ± 0.2 | 2.2 ± 0.2 | 1.9 ± 0.2 | .148 |
| 22:5n-6 | 1.4 ± 0.2$^{a}$ | 3.0 ± 0.2$^{d}$ | 2.4 ± 0.3$^{c}$ | 2.0 ± 0.2$^{b}$ | .259 |
| ∑n-6 PUFA | 11.7 ± 0.5$^{a}$ | 16.9 ± 0.6$^{c}$ | 13.9 ± 0.5$^{b}$ | 14.1 ± 0.3$^{b}$ | .249 |
| 20:5n-3 | 2.4 ± 0.4$^{c}$ | 0.8 ± 0.1$^{a}$ | 1.1 ± 0.1$^{ab}$ | 1.4 ± 0.2$^{b}$ | −.462 |
| 22:5n-3 | 7.8 ± 0.4$^{b}$ | 3.2 ± 0.4$^{a}$ | 3.7 ± 0.4$^{a}$ | 3.4 ± 0.3$^{a}$ | −.750** |
| 22:6n-3 | 28.9 ± 1.3$^{b}$ | 27.7 ± 0.3$^{b}$ | 27.2 ± 1.3$^{b}$ | 22.0 ± 2.4$^{a}$ | −.801** |
| ∑n-3 PUFA | 39.4 ± 2.1$^{c}$ | 32.1 ± 0.6$^{b}$ | 32.0 ± 1.3$^{b}$ | 27.1 ± 1.9$^{a}$ | −.898** |
| ∑PUFA | 52.8 ± 1.4$^{c}$ | 50.7 ± 0.7$^{bc}$ | 48.3 ± 0.9$^{b}$ | 44.0 ± 2.0$^{a}$ | −.927** |
| ∑n-3 LC-PUFA | 39.3 ± 2.0$^{c}$ | 31.9 ± 0.6$^{b}$ | 32.0 ± 1.3$^{b}$ | 26.8 ± 2.0$^{a}$ | −.896** |
| n-3/n-6 | 3.4 ± 0.3$^{c}$ | 1.9 ± 0.1$^{a}$ | 2.3 ± 0.2$^{b}$ | 1.9 ± 0.1$^{a}$ | −.714** |
| PIn | 334.0 ± 13.7$^{c}$ | 311.2 ± 3.8$^{c}$ | 301.9 ± 8.7$^{b}$ | 257.3 ± 16.5$^{a}$ | −.903** |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). DMA = dimethyl acetal; LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

CL increasing between 3- and 11-month-old animals, a time during which the fish doubled their body weight. Rapid growth phases are linked to a sustained high metabolic activity and, thus, with accelerated mitochondrial metabolism (33). In this situation, higher CL would be necessary and so the increased levels of CL in mitochondrial

Table 4. Fatty Acid Composition (percentage of total fatty acids) of Phosphatidylserine of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Mo | 7 Mo | 11 Mo | 14 Mo | *r* |
|---|---|---|---|---|---|
| 16:0 | 6.8 ± 0.8 | 7.3 ± 0.8 | 8.1 ± 0.6 | 7.4 ± 0.9 | .332 |
| 18:0 | 29.1 ± 0.1$^{b}$ | 31.3 ± 1.9$^{bc}$ | 24.9 ± 1.8$^{a}$ | 34.9 ± 2.3$^{c}$ | .375 |
| ∑Saturated | 38.0 ± 0.7$^{b}$ | 40.2 ± 1.5$^{b}$ | 35.3 ± 1.0$^{a}$ | 44.4 ± 1.2$^{c}$ | .515 |
| 16:1n-7 | 2.0 ± 0.4$^{a}$ | 2.7 ± 0.4$^{ab}$ | 4.1 ± 0.6$^{c}$ | 3.2 ± 0.4$^{b}$ | .619* |
| 18:1n-9 | 3.7 ± 0.2$^{a}$ | 12.3 ± 1.2$^{b}$ | 12.4 ± 0.7$^{b}$ | 15.7 ± 0.9$^{c}$ | .881** |
| 24:1n-9 | 1.6 ± 0.3 | 2.1 ± 0.7 | 1.3 ± 0.4 | 1.3 ± 0.7 | −.309 |
| ∑Monounsaturated | 11.3 ± 0.6$^{a}$ | 17.6 ± 0.9$^{b}$ | 18.6 ± 1.5$^{b}$ | 21.2 ± 0.8$^{c}$ | .915** |
| 18:2n-6 | 1.8 ± 0.5 | 1.8 ± 0.4 | 1.7 ± 0.2 | 2.1 ± 0.2 | .255 |
| 20:4n-6 | 3.2 ± 1.7 | 2.2 ± 0.1 | 1.9 ± 0.4 | 1.5 ± 0.1 | −.648* |
| 22:4n-6 | 2.9 ± 0.2$^{a}$ | 4.1 ± 0.2$^{b}$ | 3.3 ± 0.4$^{a}$ | 3.4 ± 0.3$^{a}$ | .110 |
| 22:5n-6 | 2.0 ± 0.4$^{a}$ | 3.8 ± 0.2$^{b}$ | 2.6 ± 0.3$^{a}$ | 2.1 ± 0.3$^{a}$ | −.247 |
| ∑n-6 PUFA | 11.3 ± 2.4 | 13.1 ± 0.3 | 10.8 ± 0.8 | 10.6 ± 0.5 | −.394 |
| 20:5n-3 | 1.0 ± 0.2 | 0.4 ± 0.0 | 0.6 ± 0.0 | 0.6 ± 0.1 | −.221 |
| 22:5n-3 | 11.0 ± 0.5$^{b}$ | 4.9 ± 0.5$^{a}$ | 4.8 ± 0.4$^{a}$ | 4.8 ± 0.4$^{a}$ | −.743** |
| 22:6n-3 | 26.1 ± 2.0$^{bc}$ | 22.5 ± 2.3$^{b}$ | 27.0 ± 2.8$^{c}$ | 16.3 ± 0.5$^{a}$ | −.651* |
| ∑n-3 PUFA | 38.3 ± 2.5$^{d}$ | 28.2 ± 2.0$^{b}$ | 32.9 ± 2.5$^{c}$ | 22.5 ± 1.1$^{a}$ | −.788** |
| ∑PUFA | 50.7 ± 0.2$^{d}$ | 42.2 ± 1.7$^{b}$ | 46.0 ± 1.5$^{c}$ | 34.4 ± 0.6$^{a}$ | −.843** |
| ∑n-3 LC-PUFA | 38.1 ± 2.3$^{d}$ | 28.0 ± 1.8$^{b}$ | 32.5 ± 2.4$^{c}$ | 22.2 ± 1.0$^{a}$ | −.799** |
| n-3/n-6 | 3.5 ± 1.1$^{b}$ | 2.2 ± 0.2$^{a}$ | 3.1 ± 0.5$^{ab}$ | 2.1 ± 0.2$^{a}$ | −.471 |
| PIn | 325.0 ± 10.3$^{d}$ | 267.8 ± 16.5$^{b}$ | 296.4 ± 15.7$^{c}$ | 206.5 ± 5.4$^{a}$ | −.822** |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

inner membrane are consistent with the existing paradigm. Rapid growth has also been correlated with an increased level of intracellular oxidative stress (23,34,35), along with decreased repair machinery (36). Under these conditions, mitochondrial molecules have been reported to suffer increasing deterioration that eventually can lead to the impairment of cellular bioenergetic functions, increased oxidative stress, and attenuation of stress responses (1). In a previous study performed on 1-, 4-, and 7-month-old *N. rachovii*, Hsu and colleagues (19) reported a progressive increase in lipid peroxidation and protein damage as along with decreased antioxidant activities with age. Our data showed a loss of CL content in *N. rachovii* mitochondrial membranes between 11- and 14-month-old fish. It could be suggested that this loss could be one of the first signs of damage caused by high oxidative stress in mitochondrial membranes and that peroxidation of CL could lead to the age-related decline in mitochondrial function reported in a variety of tissues (37,38). The mitochondrial membranes of *N. rachovii* also showed increased SM content between 11- and 14-month-old fish. An accumulation of SM with age has also been reported in several tissues in mammals (39–41) and in brain mitochondria of rainbow trout (38). This accumulation could have a protective role because SM has membrane-rigidifying properties that retard the lateral propagation of oxidative reactions through mitochondrial membranes (14). However, SM is also an important precursor for many signaling molecules, some associated with apoptosis, the terminal process occurring in cells with dysfunctional mitochondria (16). Increased SM content in cell membranes along with high oxidative stress has been reported to induce cell dysfunction or death by altering mitochondrial function (15). Mitochondrial membrane PL composition also showed a progressive decrease in PC content between 3- and 14-month-old fish and increased PS between 3- and 7-month-old animals. Apart from affecting PL-specific functions, changes in the proportions of individual PL classes may lead to altered charge distribution across the membrane, membrane permeability properties, catalytic activities of specific enzymes, and electron transport chain function (42).

Individual PL from whole *N. rachovii* mitochondrial membranes showed characteristic fatty acid compositions that are likely related to their specific roles in membrane fluidity and function (43,44). Fatty acid compositions of individual PL of mitochondria were influenced significantly by age. Saturated fatty acid content was generally increased in all PL classes, and monounsaturated fatty acid was increased in PC, PE, and PS, which represented the major PL components of mitochondrial membranes in *N. rachovii*. Concomitantly, n-6 PUFA content decreased in all PL classes (except PS) and n-3 PUFA decreased in PC, PE, and PS. These changes were similar to those reported in total lipid of juvenile, adult, and senescent *N. korthausae* in a previous study (32) and represent a major perturbation in the ratio of saturated fatty acid to unsaturated fatty acids in membranes that will have important implications for mitochondrial and tissue functions as reported in neurological diseases (41). The PIn decreased in the major PL classes, mainly due to decreased DHA, the fatty acid

Table 5. Fatty Acid Composition (percentage of total fatty acids) of Sphingomyelin of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Months | 7 Months | 11 Months | 14 Months | *r* |
|---|---|---|---|---|---|
| 14:0 | 2.8 ± 0.7[a] | 4.7 ± 0.8[ab] | 3.9 ± 0.4[ab] | 5.2 ± 1.0[b] | 0.620* |
| 15:0 | 2.4 ± 0.4 | 1.9 ± 0.3 | 2.3 ± 0.3 | 1.9 ± 0.2 | –0.357 |
| 16:0 | 28.4 ± 3.3[a] | 35.0 ± 1.6[b] | 35.8 ± 1.3[b] | 34.9 ± 2.9[b] | 0.587* |
| 18:0 | 10.8 ± 1.5[b] | 7.2 ± 0.6[a] | 8.0 ± 0.8[a] | 8.0 ± 0.8[a] | –0.472 |
| 20:0 | 0.7 ± 0.1[a] | 2.3 ± 0.2[b] | 1.8 ± 0.3[b] | 2.0 ± 0.6[b] | 0.533* |
| 22:0 | 1.2 ± 0.3[a] | 7.8 ± 0.8[c] | 6.1 ± 0.3[b] | 7.7 ± 1.1[c] | 0.687** |
| ∑saturated | 46.3 ± 2.4[a] | 58.8 ± 2.9[b] | 58.0 ± 1.3[b] | 59.8 ± 2.0[b] | 0.730** |
| 16:1n-7 | 5.5 ± 0.7[b] | 3.0 ± 1.2[ab] | 4.2 ± 2.3[ab] | 2.0 ± 0.3[a] | –0.615* |
| 18:1n-9 | 8.6 ± 1.1[b] | 3.9 ± 1.6[a] | 3.9 ± 1.4[a] | 2.8 ± 0.7[a] | –0.758** |
| 18:1n-7 | 2.8 ± 0.1[b] | 1.3 ± 0.3[a] | 1.7 ± 1.1[ab] | 0.7 ± 0.0[a] | –0.693** |
| 22:1n-9 | 0.0 ± 0.0[a] | 2.1 ± 1.3[b] | 2.3 ± 0.2[b] | 3.0 ± 0.4[b] | 0.772** |
| 24:1n-9 | 3.4 ± 0.1[a] | 9.8 ± 0.7[b] | 10.0 ± 0.6[b] | 10.4 ± 1.8[b] | 0.759** |
| ∑monounsaturated | 22.1 ± 1.9 | 21.1 ± 2.1 | 23.5 ± 2.6 | 19.9 ± 1.3 | –0.253 |
| 18:2n-6 | 4.1 ± 0.5[b] | 1.4 ± 0.8[a] | 0.8 ± 0.1[a] | 0.8 ± 0.2[a] | –0.804** |
| 18:3n-6 | 3.1 ± 0.4[c] | 1.6 ± 0.2[b] | 2.7 ± 1.4[bc] | 0.8 ± 0.2[a] | –0.374 |
| 22:4n-6 | 0.4 ± 0.1 | 2.0 ± 0.2 | 1.8 ± 0.4 | 1.8 ± 0.5 | 0.258 |
| 22:5n-6 | 5.6 ± 0.8[c] | 0.5 ± 0.1[a] | 0.2 ± 0.3[a] | 1.8 ± 0.5[c] | –0.642* |
| ∑n-6 PUFA | 14.8 ± 0.9[b] | 6.7 ± 1.1[a] | 6.6 ± 1.4[a] | 7.1 ± 0.8[a] | –0.681** |
| 18:4n-3 | 1.8 ± 0.2[c] | 0.5 ± 0.2[a] | 1.1 ± 0.1[b] | 0.9 ± 0.2[ab] | –0.457 |
| 20:5n-3 | 1.2 ± 0.0 | 0.5 ± 0.1 | 1.0 ± 0.1 | 0.7 ± 0.3 | –0.122 |
| 22:5n-3 | 3.1 ± 0.3[b] | 1.5 ± 0.3[a] | 2.4 ± 0.6[b] | 1.6 ± 0.4[a] | –0.505 |
| 22:6n-3 | 4.1 ± 0.4[a] | 6.4 ± 0.6[b] | 4.5 ± 0.0[a] | 7.7 ± 1.4[b] | 0.630* |
| ∑n-3 PUFA | 12.0 ± 0.3[b] | 8.8 ± 0.5[a] | 9.2 ± 0.7[a] | 11.5 ± 1.3[b] | 0.041 |
| ∑PUFA | 31.6 ± 2.9[b] | 20.1 ± 4.2[a] | 18.5 ± 1.4[a] | 20.4 ± 0.8[a] | –0.647* |
| ∑n-3 LC-PUFA | 10.1 ± 0.2[b] | 8.3 ± 0.5[a] | 8.1 ± 0.7[a] | 10.7 ± 1.4[b] | 0.239 |
| n-3/n-6 | 0.8 ± 0.0[a] | 1.4 ± 0.2[b] | 1.4 ± 0.2[b] | 1.7 ± 0.4[b] | 0.755** |
| PIn | 133.5 ± 10.8[c] | 93.5 ± 7.8[ab] | 86.3 ± 6.5[a] | 107.5 ± 6.8[b] | –0.416 |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

most prone to peroxidation. Considering that several of these changes occurred between 11- and 14-month-old fish, after growth in these animals had ceased, it could be a consequence or related to the effects of the previous high growth rate and the rapid attainment of an adult body size, which has been reported to produce several negative side effects in animals and to have important repercussions over a species' life span (33). These effects would be associated with a sustained increased level of intracellular oxidative stress along with decreased repair systems (36), which would cause damage to mitochondrial molecules, starting with those particularly susceptible to oxidation such as linoleic acid (very high in CL) and, particularly, DHA (high in PC, PE, and PS). PUFA would be the first target of oxidative radicals and would contribute to their propagation through the membrane and further to other mitochondrial molecules, eventually leading to mitochondrial dysfunction (23,38).

Summarizing, although the mitochondrial isolation from whole animals performed in the present study could constitute a limitation to conclude about specific tissues, the present results, along with other data showing an increase in lipid and protein oxidation and a reduction in antioxidant activities in whole *N. rachovii* with age, suggest increasing oxidative stress and damage to mitochondrial lipids during the first months of this species' life cycle, which could likely determine their short life span. Following the membrane pacemaker theory of animal metabolism (3,45), lipids would be among the first molecules affected by mitochondrial free radicals, and lipid peroxidation could be the propagator of oxidative damage reactions. Both mitochondrial membrane PL class and PL fatty acid compositions changed in *N. rachovii* with age, which could considerably alter their properties as the major constituents of mitochondrial membranes. Particularly important were the changes observed in CL and SM as these PL have been proposed as mediators in mitochondrial dysfunction and apoptosis as consequences of situations of high oxidative stress and aging. Also important are the changes observed in individual PL fatty acid composition as they greatly influence PL-specific properties. These data also support the use of fish of the genus *Nothobranchius* for the study of aging. Further research is necessary to understand the multiple mechanisms involved in aging and their cause–effect relationships, and *Nothobranchius* species, as vertebrate models, can be particularly useful in this effort.

Table 6. Fatty Acid Composition (percentage of total fatty acids) of Phosphatidylinositol of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Mo | 7 Mo | 11 Mo | 14 Mo | *r* |
|---|---|---|---|---|---|
| 15:0 | 0.8 ± 0.2 | 0.4 ± 0.2 | 1.2 ± 0.3 | 0.6 ± 0.1 | −.215 |
| 16:0 | 9.4 ± 0.8$^{ab}$ | 8.1 ± 0.7$^{a}$ | 13.0 ± 2.7$^{b}$ | 9.0 ± 0.7$^{ab}$ | .207 |
| 18:0 | 18.4 ± 2.1$^{a}$ | 22.0 ± 0.9$^{ab}$ | 19.7 ± 4.0$^{a}$ | 26.5 ± 2.4$^{b}$ | .598* |
| ∑Saturated | 29.8 ± 2.6$^{a}$ | 31.9 ± 0.7$^{ab}$ | 36.3 ± 4.1$^{b}$ | 37.8 ± 1.6$^{b}$ | .793** |
| 16:1n-7 | 4.6 ± 0.8 | 2.4 ± 0.4 | 4.5 ± 1.8 | 2.3 ± 0.3 | −.328 |
| 18:1n-9 | 6.9 ± 0.4$^{a}$ | 8.0 ± 1.1$^{ab}$ | 8.1 ± 0.9$^{b}$ | 7.1 ± 1.0$^{ab}$ | .336 |
| 18:1n-7 | 10.8 ± 1.2$^{b}$ | 5.1 ± 1.1$^{a}$ | 7.8 ± 2.2$^{ab}$ | 4.6 ± 0.5$^{a}$ | −.529 |
| 24:1n-9 | 1.3 ± 0.0$^{a}$ | 1.4 ± 0.2$^{a}$ | 2.4 ± 0.7$^{b}$ | 1.3 ± 0.1$^{a}$ | .203 |
| ∑Monounsaturated | 24.1 ± 1.6$^{b}$ | 17.4 ± 2.8$^{a}$ | 24.2 ± 4.8$^{ab}$ | 16.0 ± 2.3$^{a}$ | −.376 |
| 18:2n-6 | 8.0 ± 1.4$^{b}$ | 2.0 ± 0.1$^{a}$ | 2.7 ± 1.1$^{a}$ | 1.5 ± 0.1$^{a}$ | −.778** |
| 18:3n-6 | 1.2 ± 0.1 | 0.7 ± 0.1 | 1.8 ± 0.9 | 0.7 ± 0.2 | −.037 |
| 20:4n-6 | 6.6 ± 1.4$^{a}$ | 14.3 ± 1.3$^{b}$ | 9.3 ± 3.0$^{ab}$ | 10.3 ± 2.1$^{ab}$ | .200 |
| 22:4n-6 | 1.2 ± 0.0$^{a}$ | 2.2 ± 0.3$^{b}$ | 1.6 ± 0.4$^{ab}$ | 2.2 ± 0.3$^{b}$ | .553* |
| 22:5n-6 | 0.8 ± 0.2$^{a}$ | 1.7 ± 0.4$^{b}$ | 1.0 ± 0.4$^{ab}$ | 1.7 ± 0.1$^{b}$ | .568* |
| ∑n-6 PUFA | 19.0 ± 0.6$^{a}$ | 22.4 ± 1.4$^{b}$ | 17.6 ± 1.5$^{a}$ | 17.6 ± 1.8$^{a}$ | −.448 |
| 20:5n-3 | 1.9 ± 0.3$^{b}$ | 1.1 ± 0.1$^{a}$ | 1.4 ± 0.2$^{a}$ | 1.5 ± 0.1$^{a}$ | −.342 |
| 22:5n-3 | 6.7 ± 0.4$^{b}$ | 3.5 ± 0.7$^{a}$ | 3.8 ± 0.3$^{a}$ | 4.2 ± 0.1$^{a}$ | −.633* |
| 22:6n-3 | 13.8 ± 3.0$^{a}$ | 22.1 ± 3.0$^{b}$ | 13.0 ± 6.0$^{ab}$ | 21.7 ± 3.1$^{b}$ | .252 |
| ∑n-3 PUFA | 23.4 ± 4.4 | 27.4 ± 3.6 | 19.4 ± 5.6 | 27.7 ± 3.2 | .071 |
| ∑PUFA | 46.1 ± 1.8$^{ab}$ | 50.7 ± 2.3$^{b}$ | 39.5 ± 5.1$^{a}$ | 46.1 ± 1.7$^{ab}$ | −.277 |
| ∑n-3 LC-PUFA | 22.7 ± 3.8 | 27.1 ± 3.7 | 18.7 ± 5.8 | 27.6 ± 3.1 | .092 |
| n-3/n-6 | 1.2 ± 0.3 | 1.2 ± 0.2 | 1.1 ± 0.3 | 1.6 ± 0.4 | .330 |
| PIn | 221.2 ± 21.5$^{ab}$ | 290.9 ± 25.2$^{b}$ | 203.7 ± 49.1$^{a}$ | 275.9 ± 18.1$^{ab}$ | .146 |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

Table 7. Fatty Acid Composition (percentage of total fatty acids) of Cardiolipin of 3-, 7-, 11-, and 14-Month-Old *Nothobranchius rachovii*

| Fatty Acid | 3 Mo | 7 Mo | 11 Mo | 14 Mo | *r* |
|---|---|---|---|---|---|
| 14:0 | 1.3 ± 0.4$^{ab}$ | 0.6 ± 0.0$^{a}$ | 1.1 ± 0.1$^{ab}$ | 1.6 ± 0.4$^{b}$ | .285 |
| 15:0 | 1.2 ± 0.3$^{ab}$ | 0.8 ± 0.2$^{a}$ | 1.1 ± 0.1$^{ab}$ | 1.5 ± 0.5$^{b}$ | .288 |
| 16:0 | 11.7 ± 0.7$^{ab}$ | 7.2 ± 0.7$^{a}$ | 11.0 ± 0.7$^{ab}$ | 14.4 ± 4.9$^{b}$ | .352 |
| 18:0 | 5.0 ± 0.5$^{a}$ | 11.1 ± 3.0$^{b}$ | 4.2 ± 0.3$^{a}$ | 9.1 ± 1.4$^{b}$ | .195 |
| ∑Saturated | 19.7 ± 1.2$^{a}$ | 20.2 ± 3.5$^{a}$ | 17.9 ± 1.0$^{a}$ | 27.8 ± 5.7$^{b}$ | .472 |
| 16:1n-7 | 8.2 ± 0.8 | 7.3 ± 1.1 | 8.1 ± 1.2 | 6.4 ± 0.8 | −.435 |
| 18:1n-9 | 8.9 ± 0.7 | 10.9 ± 0.7 | 9.7 ± 0.6 | 11.1 ± 3.1 | .414 |
| 18:1n-7 | 12.6 ± 1.9 | 14.9 ± 1.5 | 14.8 ± 1.2 | 12.4 ± 2.9 | .024 |
| 24:1n-9 | 1.5 ± 0.6$^{a}$ | 1.6 ± 0.2$^{a}$ | 2.4 ± 0.5$^{a}$ | 4.0 ± 1.0$^{b}$ | .822** |
| ∑Monounsaturated | 31.9 ± 1.4 | 35.2 ± 2.4 | 35.9 ± 1.0 | 34.9 ± 2.8 | .544* |
| 18:2n-6 | 21.5 ± 1.6$^{c}$ | 16.2 ± 2.8$^{b}$ | 20.5 ± 2.1$^{bc}$ | 10.5 ± 2.8$^{a}$ | −.652* |
| 18:3n-6 | 2.0 ± 1.0 | 0.8 ± 0.1 | 1.8 ± 0.3 | 1.6 ± 0.5 | −.051 |
| 20:2n-6 | 1.8 ± 0.4 | 1.4 ± 0.2 | 1.6 ± 0.1 | 1.6 ± 0.5 | −.141 |
| 20:4n-6 | 1.1 ± 0.2$^{a}$ | 6.8 ± 2.0$^{b}$ | 1.5 ± 0.1 | 2.3 ± 0.8 | −.035 |
| 22:5n-6 | 0.4 ± 0.3$^{a}$ | 1.2 ± 0.2$^{b}$ | 1.3 ± 0.3$^{b}$ | 1.5 ± 0.4$^{b}$ | .804** |
| ∑n-6 PUFA | 28.1 ± 0.5$^{b}$ | 28.4 ± 1.6$^{b}$ | 28.0 ± 2.4$^{b}$ | 19.4 ± 2.9$^{a}$ | −.674** |
| 18:3n-3 | 1.4 ± 0.2$^{c}$ | 1.2 ± 0.3$^{bc}$ | 0.9 ± 0.1$^{ab}$ | 0.6 ± 0.1$^{a}$ | −.865** |
| 20:5n-3 | 0.9 ± 0.2$^{ab}$ | 0.6 ± 0.1$^{a}$ | 0.6 ± 0.0$^{a}$ | 1.3 ± 0.1$^{b}$ | .178 |
| 22:5n-3 | 3.5 ± 0.2$^{c}$ | 2.5 ± 0.3$^{a}$ | 2.6 ± 0.1$^{ab}$ | 2.9 ± 0.1$^{b}$ | −.458 |
| 22:6n-3 | 8.5 ± 1.2 | 9.1 ± 0.4 | 8.9 ± 0.5 | 9.3 ± 3.2 | .183 |
| ∑n-3 PUFA | 15.7 ± 1.1 | 14.2 ± 0.3 | 13.9 ± 1.1 | 14.9 ± 3.0 | −.249 |
| ∑PUFA | 48.4 ± 1.7$^{b}$ | 44.6 ± 1.8$^{b}$ | 46.2 ± 0.2$^{b}$ | 37.3 ± 5.0$^{a}$ | −.732** |
| ∑n-3 LC-PUFA | 13.6 ± 1.3 | 12.8 ± 0.6 | 12.5 ± 0.9 | 14.1 ± 3.0 | .028 |
| n-3/n-6 | 0.6 ± 0.0$^{a}$ | 0.5 ± 0.0$^{a}$ | 0.5 ± 0.0$^{a}$ | 0.8 ± 0.1$^{b}$ | .572* |
| PIn | 149.6 ± 6.0 | 160.0 ± 9.9 | 148.2 ± 4.3 | 150.7 ± 29.4 | −.062 |

*Notes*: Results are presented as mean ± *SD* ($n = 4$). Different superscript letters represent significant differences between age groups as determined by one-way analysis of variance ($p < .05$). Right column includes values for Pearson correlation coefficient ($r$) calculated for each fatty acid or index and age. Significant values are indicated with asterisks (*$p < .05$, **$p < .01$). LC-PUFA = long-chain polyunsaturated fatty acids; PIn = peroxidation index; PUFA = polyunsaturated fatty acids. Fatty acids representing less than 1% of total fatty acids are not shown.

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