Almaida-Pagan PF, de Costa J, Mendiola P & Tocher DR (2012) Changes in tissue and mitochondrial membrane composition during rapid growth, maturation and aging in rainbow trout, Oncorhynchus mykiss, *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology*, 161 (4), pp. 404-412.

This is the peer reviewed version of this article

Changes in tissue and mitochondrial membrane composition during rapid growth, maturation and aging in rainbow trout, *Oncorhynchus mykiss*

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Running title: Mitochondrial lipid composition in rapid growth and aging of fish

Abstract

Membrane compositions, particularly of mitochondria, could be critical factors in the mechanisms of growth and aging processes, especially during phases of high oxidative stress that result in molecular damage. In the present study, liver and mitochondrial membrane phospholipid (PL) compositions were analyzed in rainbow trout during its four first years of life, a period characterized by rapid growth and high oxidative stress. Specifically, farmed fish of three ages (1-, 2- and 4-years) were studied, and PL compositions of whole liver and liver mitochondria, and fatty acid compositions of individual PL classes were determined. Liver mitochondrial membranes showed a PL composition different to that of the whole tissue suggesting adaptation of cell and subcellular membranes to specific functions. Individual PL had characteristic fatty acid compositions that were similar in whole liver and mitochondrial membranes. Whole liver and mitochondria showed increased lipid peroxidation with age along with changes in membrane PL fatty acid compositions. Most PL classes showed similar changes in fatty acid composition among the age groups, with reduced proportions of docosahexaenoic acid (DHA) and, generally, concomitantly increased levels of monounsaturated fatty acids, which together resulted in reduced peroxidation index (PIn). However, total polyunsaturated fatty acid (PUFA) content did not change significantly with age due to increased eicosapentaenoic acid, docosapentaenoic acid and, in most PL, increased n-6 PUFA. These results suggest there may be oxidation of PL DHA with compensatory mechanisms to maintain membrane fluidity and function. However, modification of fatty acid composition of specific PLs, such as cardiolipin, could affect the electron transport chain efficiency and propagate the oxidative reaction throughout the cell. In addition, both the content and fatty acid composition of sphingomyelin, which has been suggested as a possible mediator of cell dysfunction and apoptosis, changed with age differently to the other PL classes. Moreover, these changes showed different trends between mitochondria and whole liver. These data suggest there is marked oxidative stress associated with rapid growth and maturation in rainbow trout. Changes observed in membrane lipids point to their possible participation in the processes involved in this species response to oxidative stress and damage accumulation rate.

Keywords: Aging, Cardiolipin, Fish, Growth, Mitochondria, Oxidative stress, Phospholipid, Sphingomyelin.

1. Introduction

Several studies have reported positive correlations between growth rate and levels of oxidative stress in animals (Alonso-Alvarez 2007 and references therein). The combination of a high growth rate and the rapid attainment of a large body size has several negative side-effects in animals including reduced immunological competence, depletion of energy reserves and decreased life-span (Inness and Metcalfe 2008). Effects of rapid growth on oxidative stress are mediated mainly by an increase in metabolic rate and the consequent enhancing of free radical production by mitochondria, but also by a diversion of resources into anabolism and away from repairing oxidative damage to cell molecules (Almroth *et al.* 2010). Free radicals, mainly reactive oxygen species (ROS) produced by the mitochondrial electron transport chain (ETC), have been proposed as key factors causing oxidative stress and molecular damage with age in animals (Barja 2004; Balaban *et al.* 2005; Sanz *et al.* 2006), and mitochondrial dysfunction as a contributor influencing the timing and severity of such deterioration (Shigenaga *et al.* 1994; Paradies *et al.* 2010a,b).

Although ROS damage affects all cell macromolecules, lipid peroxidation is quantitatively the main oxidative process in tissues due the high sensitivity to oxidation of polyunsaturated fatty acids (PUFA), which are essential constituents of cell membrane phospholipids (PL) (Bielski *et al.* 1983). Lipid peroxidation produces several oxidized fatty acid derivatives that propagate oxidative damage by attacking other membrane components, lipids, proteins and nucleic acids (Sanz *et al.* 2006), and it could therefore be suggested that lipid peroxidation, mainly that of mitochondrial membranes, may be the primary process associated with periods of high oxidative stress. Moreover, it has been recently suggested that lipid peroxidation derivatives could also have specific signalling roles inducing adaptive responses driven to decrease oxidative damage and improve antioxidant defences (Pamplona and Barja, 2011), membrane composition acting as a pacemaker of processes related with oxidative damage accumulation and aging.

Mitochondrial membranes have a particular lipid composition constituted by specific PL in the vicinity of the ETC components, which has been suggested to be related with the role of mitochondria in oxygen consumption (Hoch 1992). Besides phosphoacylglycerols, major components of all

membranes, mitochondrial membranes uniquely contain cardiolipin (CL), a PL with unusual structure that constitutes 12% of mitochondrial PLs in mammals (Barceló-Coblijin and Murphy 2008). Thus, CL is a key molecule for mitochondrial function, participating in many processes including regulation of electron transport and efficiency of oxidative phosphorylation (Paradies *et al.* 2002; 2011). As it is located in the mitochondrial inner membrane near to the site of ROS production and it has a high content of PUFA, CL is particularly prone to peroxidation and the effects of mitochondrial ROS. Another potentially important PL is sphingomyelin (SM), a component of all cell membranes that has also been suggested as a mediator of aging and determinant of life-span (Cutler and Mattson 2011). SM not only has membrane-rigidifying properties, which retard the lateral propagation of free radicals (Subbaiah *et al.* 1999), but is also a precursor for many signalling molecules, some associated with apoptosis, the last process taking place in cells with dysfunctional mitochondria (Hannum and Obeid 1997). The specific roles of individual PL classes are associated with characteristic fatty acid compositions that confer specific properties related to membrane fluidity and functions (Zabelinskii *et al.* 1999). Therefore, effects of oxidative stress on cell membranes could include not only changes in PL class composition, but also alterations in PL fatty acid compositions that would modify their molecular properties and therefore, their roles in membrane functions.

The overall aim of the present study was to characterize changes in rainbow trout liver membrane PL with rapid growth and age, focussing on alterations to PL composition or specific individual PL fatty acid compositions that may be critical in the modulation of mitochondria function during periods of high oxidative stress. Specifically, we investigated trout in their first four years of life, a period during which this species undergoes rapid growth. Both whole liver and liver mitochondria were analyzed, as liver is a primary metabolic tissue with high metabolism and a high mitochondrial density. Rainbow trout (*Oncorhynchus mykiss*) is a potentially useful vertebrate model not only because it presents a rapid growth phase, but also because it is a well-studied species, widely reared in European countries, and its age can be easily monitored. Furthermore, rainbow trout exhibits gradual senescence (Almroth *et al.* 2010) and, along with other species of salmonids, it has been used previously in studies of oxidative stress and mitochondrial function (Otto and Moon 1996; Zabelinskii *et al.* 1999; Kraffe *et al.* 2007; Østbye *et al.* 2011).

2. Materials and methods

2.1. Experimental fish and sampling

This experiment was performed on stock rainbow trout (*Oncorhynchus mykiss*) of three ages (1-, 2- and 4-years), all with the same genetic origin and maintained on the same rearing and feeding conditions in the freshwater aquarium facilities of the Institute of Aquaculture, University of Stirling. Fish were fed commercial feed formulated to contain 50% protein and 19% or 22% fat for younger (1-2 years) or older (4 year-old) fish (Skretting, Northwich, UK). Fatty acid compositions of the feeds were essentially similar (Table 1). Fish were anesthetized in 10% benzocaine, killed by a blow to the head, weight and length measured, and livers dissected. Whole livers were homogenized by using a blender to produce a pate that was used as source material for analyses of both whole tissue and preparation of mitochondria. Three replicate samples of each age group were collected for lipid and fatty acid analysis and lipid peroxidation status (TBARS content). In order to obtain sufficient material for all the required analyses, 1- and 2-year old trout samples consisted of livers pooled from 21 (3 pools of 7) and 12 (3 pools of 4) fish, respectively. Samples from 4-year old trout were livers from three individuals. Lipid extractions were performed on fresh samples of whole liver pate or mitochondrial preparations. Fish were treated in accordance with British national ethical requirements established by the UK Government Home Office and guidelines determined by the Animals (Scientific Procedures) Act 1986.

2.2. Mitochondria isolation

Approximately 2 g of liver pate was homogenized in 8 ml ice-cold sucrose buffer (0.4M phosphate buffer pH 7.4, 0.25M sucrose, 0.15M KCl, 40mM KF and 1mM N-acetyl-cysteine) using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). Homogenates were then centrifuged at 600 x *g* for 6 min and the pellet discarded (cell/nuclei debris). Supernatants were then centrifuged at 6,800 x *g* for 10 min and the resulting pellet (mitochondrial fraction) used for lipid extraction. To verify that pellets were highly enriched with mitochondria, a portion was fixed in 2.5% glutaraldehyde

in 0.1M cacodylate buffer overnight at 4ºC, and then processed as specified by Rajapakse *et al.* (2001) prior to analysis by transmission electron microscopy (Tecnai^{TM} G^2 Spirit BioTWIN, FEI Europe, Eindhoven, The Netherlands).

2.3. Lipid extraction and phospholipid class composition

Total lipid extracts from feeds, liver tissue and mitochondria were obtained after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch *et al.* (1957). Approximately 1 g of ground feed or liver tissue pate was homogenized in 20 ml of ice-cold chloroform/methanol (2:1, by vol.) using an Ultra-Turrax tissue disrupter followed by addition of 5 ml of 0.88% (w/v) KCl, mixing and layers allowed to separate on ice for 1 h. The upper non-lipid layer was aspirated and the lower lipid layer was evaporated under a stream of oxygen-free nitrogen. The lipid content was determined gravimetrically after drying overnight in a vacuum desiccator. Essentially the same procedure was used for the liver mitochondria preparations although the reagent volumes were adapted (5 ml of chloroform/methanol, 2:1 and 1 ml of 0.88% KCl). All lipid extracts were stored at -20 ºC under a N_2 atmosphere prior to analysis.

Phospholipid classes were separated by high-performance thin-layer chromatography (HPTLC) using 10 x 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 vol.) as solvent system (Olsen and Henderson 1989). The lipid classes were visualized by charring at 160 ºC for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16) (Henderson and Tocher 1992). Scanned images were recorded automatically and analyzed by computer using winCATS (Planar Chromatography Manager, version 1.2.0).

2.4. Phospholipid fatty acid composition

Individual phospholipid classes of whole liver and liver mitochondria were separated by preparative-TLC, using silica gel plates (20 x 20 cm) (VWR) and the solvent system as above. Individual phospholipid bands 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) BHT, and visualization under UV light (UVGL-58 Minerallight® Lamp, Ultraviolet Prod. Inc., Calif., USA). Each phospholipid class was scraped from the plate into a test tube and subjected directly (on silica) to acid-catalyzed transmethylation at 50ºC following addition of 2 ml of 1% (v/v) sulphuric acid in methanol in order to obtain the fatty acid methyl esters (FAME) (Christie 2003). Similarly, FAME were also produced by acid-catalyzed transmethylation of samples of total lipid from feeds. FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection at 50 ºC. Hydrogen was used as carrier gas and temperature programming was from 50 ºC to 150 ºC at 40 ºC min^{-1} and then to 230 ºC at 2.0 ºC min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman 1980; Tocher and Harvie 1988). Data were collected and processed using Chromcard for Windows (version 1.19).

2.5. Measurement of thiobarbituric acid reactive substances (TBARS)

Approximately 1 mg of total lipid extracts (liver tissue and mitochondria) was used for the measurement of TBARS using an adaptation of the protocol of Burk *et al.* 1980. Briefly, 50µl of 0.2% (w/v) BHT in ethanol was added to the sample followed by 0.5 ml of 1% (w/v) TBA and 0.5 ml 10% (w/v) TCA, both solutions freshly prepared. The reagents were mixed in a stoppered test tube and heated at 100 ºC for 60 min. After cooling, possible floaters were removed by centrifugation at 2000 x *g*, and fluorescence in the supernatant determined in a spectrophotometer (Uvikon 860, Kontron Instruments, St. Albans, U.K.) at 532 nm against a blank sample. The concentration of TBARS, expressed as nmol/ g of lipid, was calculated using the extinction coefficient 0.156 μM^{-1} cm^{-1}.

2.6. Indexes and statistical analysis

Condition factor (K) was calculated using the formula: K = (weight/(length)^3) x 100. For peroxidation index (PIn) the formula was: PIn = 0.025 × (% monoenoics) +1 × (% dienoics) + 2 × (% trienoics) + 4 × (% tetraenoics) + 6 × (% pentaenoics) + 8 × (% hexaenoics) (Witting and Horwitt 1964). The LC-PUFA index corresponds with the sum of long-chain polyunsaturated fatty acids (LC-PUFA, fatty acids with 20 or more carbons and 3 or more double bonds). Results are presented as mean ± SD (n = 3). Data were checked for homogeneity of variances by the Levene´s test and, where necessary, arc-sin transformed before further statistical analysis. One-way ANOVA was performed to determine statistical significance of differences between age groups for each fatty acid, group of fatty acids, index or TBARS content, and Tukey´s post hoc test was used for multiple comparisons when pertinent. For comparisons between whole liver and liver mitochondria, the Student t-test was used. All statistical analyses were performed using SPSS Statistical Software System version 15.0 (SPSS Inc, Chicago, USA). Differences were regarded as significant when $P < 0.05$ (Zar 1999).

3. Results

3.1. Biometric measurements

The biometric data of the trout used in the study are presented in Table 2. The increase in weights between the age groups were 3.0-fold from 1 to 2 years, and 25.9-fold from 2 to 4 years. The increase in lengths between the age groups were 1.4-fold from 1 to 2 years. and 2.9-fold from 2 to 4 years. Condition factor (K) was similar among the age groups.

3.2. *Phospholipid class composition of liver and liver mitochondria*

Table 3 shows phospholipid class composition of whole liver and liver mitochondria from rainbow trout. In both liver and mitochondria, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) constituted more than 60% of total phospholipids. Among the remaining phospholipid classes phosphatidylinositol (PI) was the most abundant followed by CL, phosphatidylserine (PS) and

213 sphingomyelin (SM). Several differences were found between liver tissue and mitochondrial
214 membrane phospholipid compositions. The percentage of total phospholipid was higher in
215 mitochondria compared to that from liver tissue, particularly in younger fish (e.g. 83% vs. 61% in 1
216 year-old trout). Mitochondria showed higher proportions of CL and generally lower percentages of
217 PC.

218 Whereas the proportions of total phospholipids increased in whole liver, mitochondrial total
219 phospholipid content decreased significantly with age (Table 3). Some differences in the proportions
220 of the major PL, PC and PE, were observed in mitochondria but they did not correlate with age. A
221 similar pattern in PE level to that observed in mitochondria was also found in whole liver, with the
222 percentage of PE decreased between 1 and 2 year-old fish and increased between 2 and 4 year-old
223 animals. However, unlike mitochondria, liver PI, PS and SM significantly increased between 1 and 2
224 year-old fish.

225

226 *3.3. Fatty acid compositions of individual phospholipids of liver and liver mitochondria*

227 Fatty acid compositions of individual phospholipid classes from liver tissue and liver mitochondria of
228 1-, 2- and 4-year-old rainbow trout are shown in Tables 4-9. Each phospholipid class showed a
229 distinctive fatty acid profile. Thus, PC was characterized by high percentages of 16:0 and
230 docosahexaenoic acid (DHA; 22:6n-3) (Table 4), PE had high percentages of 18:1n-9 and
231 eicosapentaenoic acid (EPA; 20:5n-3) (Table 5), CL showed high levels of 16:0, 16:1n-7 and 18:2n-6
232 (Table 6), PI was characterized by high levels of 18:0 and arachidonic acid (ARA; 20:4n-6) (Table 7),
233 PS contained high 18:0 and DHA (Table 8), and SM was characterized by a very high proportion of
234 24:1n-9 (Table 9).

235 The fatty acid compositions of individual PLs were similar in liver tissue and liver mitochondria, with
236 just slight differences. In contrast, the fatty acid compositions of individual PL classes in both whole
237 liver and liver mitochondria were highly influenced by age. The effects were qualitatively similar in
238 both liver and mitochondria but, generally, quantitatively greater in mitochondria with many
239 significant differences, as described below. Thus, in mitochondria, the peroxidation index (PIn)

decreased with age in almost every PL class except SM in which it increased (Table 9). The decreased PIn was mainly caused by decreased DHA, especially marked in PC, PE and CL (11.3, 19.2 and 14.3% points, respectively) (Tables 4-6). However, the decreased DHA was accompanied by increased EPA and 22:5n-3 in all PL classes including SM. Total saturated fatty acids decreased in most PL, especially PI (9.6% points between 1 and 4 year-old trout), with the exception of SM in which saturates increased with age. The proportion of 16:0 in CL was 16 - 17 % points lower in 2-year-old animals compared to 1- and 4-year-old trout (Table 6). Total monounsaturated fatty acids increased with age in PE, PI and PS, and decreased in SM. There was a marked decrease in 24:1n-1 (14.1 % points) in SM with age, mainly between 2- and 4-year-old fish.

Liver PLs showed similar changes with age in fatty acid composition with the exception again being SM, in which there were many differences when comparing liver and mitochondria from 4-year-old trout (Table 9). The percentages of DHA (4.6 vs. 13.6%), total n-3 PUFA (8.4 vs. 21.0%) and PIn (69.2 vs. 163.8) were lower in SM of liver compared to mitochondria in 4-year-old trout. Saturated fatty acids (29.5 vs. 36.9%) were also lower in SM of liver but 24:1n-9 (43.3 vs. 22.1%) and total monounsaturated fatty acids were higher (57.5 vs. 37.5%), and EPA did not increase with age

3.4. TBARS

Lipid peroxidation in total lipid of whole liver and liver mitochondria was estimated by measuring the TBARS contents (Fig. 1). In both liver and mitochondria, the levels of TBARS significantly increased with age although the pattern was slightly different. Thus, in liver tissue, TBARS content was 26-fold higher in 4 year-old than in 1 year-old trout with the main increment produced between 1 and 2 year-old animals (increased 17-fold). In contrast, mitochondria from liver showed no increment of TBARS content between 1 and 2 year-old animals but it increased 5-fold in 4 year-old trout. The TBARS content was significantly higher in liver tissue than in mitochondria in both 2 and 4 year-old trout.

4. Discussion

Mitochondria isolated from rainbow trout liver showed a different lipid composition to that of whole liver tissue with a higher proportion of total PL although this is probably most likely the consequence of higher neutral lipid, triacylglycerol, in the liver samples. However, mitochondria also showed a different distribution of PL classes in their membranes, having a specific molecule, CL (the presence of CL in whole liver tissue being largely due to the presence of mitochondria), which is balanced mainly by lower proportions of the main PL classes, PE and PC, in liver tissue. The reciprocal relationship between CL and PC/PE has been shown previously in mammals (Tsalouhidou *et al.* 2006) and also in white muscle of salmon (Østbye *et al.* 2011). The overall different PL composition between mitochondrial and other cellular membranes reflects the different roles and specific functions of the different membranes probably also related to fundamental membrane properties including fluidity, protein binding and signalling pathways (Tocher *et al.* 2008). The fatty acid composition of the trout PLs was generally similar between liver tissue and mitochondria. This contrasts with the results obtained in rat muscle in which mitochondrial PL showed a lower level of PUFA, which the authors suggested is a result of natural selection favouring membranes that are more resistant to oxidative damage by ROS (Tsalouhidou *et al.* 2006). Recently in salmon, the same difference in PL fatty acid compositions between white muscle and muscle mitochondria was reported in fish fed diets with low and intermediate n-3 LC-PUFA contents (Østbye *et al.* 2011). However, there was less difference in PL fatty acid composition between white muscle and muscle mitochondria when the salmon were fed high EPA or high DHA diets. The diets used in the present study had LC-PUFA levels that were between those of the intermediate and high n-3 LC-PUFA diets used by Østbye *et al.* (2011) that were formulated with rapeseed oil, fish oil and EPA and DHA concentrates and so the two studies are not directly comparable.

Between the first and the fourth year the weight of the rainbow trout increased around 80-fold (from 38 to 2986 g) and their length increased 4-fold (from 14 to 60 cm) indicating considerable and rapid growth. This period of growth/maturation has been previously related with a decrease in antioxidant activities in many tissues, including liver, of rainbow trout (Otto and Moon 1996), and also in liver and brain of brown trout (Almroth *et al.* 2010). Passi *et al.* (2004) also reported an age-dependent increase in protein oxidation in muscle of rainbow trout. The results of the present study showed an

increase in lipid peroxidation with age in both liver tissue and liver mitochondria when determined by TBARS assay. The combination of the growth and lipid oxidation data suggest increased oxidative stress and decreased stress response capability in trout tissues with age during the first few years of their life. As this study used farmed fish, chosen so that age and nutritional background could be conclusively established, it was not possible to obtain individuals older than 4-years as hatcheries do not retain those animals. Therefore, considering that rainbow trout can live for more than seven years in the wild under favourable conditions, the changes observed in the present study can be considered as related specifically to a phase of rapid growth and maturation. Nevertheless, the fact that the changes were consistent with data reported in previous studies on aging supports the view that we could be observing the beginning of gradual senescence in the analyzed fish and that the rate of damage accumulation will likely increase as trout get even older. (Sohal and Weindruck 1996; Kishi *et al.* 2003; Hsu *et al.* 2008).

Most of the changes in PL class composition observed in both mitochondria and, especially liver, occurred between 1- and 2-year-old trout. In liver, these changes in PL composition paralleled the increased lipid peroxidation showed in liver between 1- and 2-year-old animals with TBARS content 17-fold higher in the older fish. However, in liver mitochondria, the main increase in lipid peroxidation occurred between 2- and 4-year-old fish, and was not reflected or associated with any major effects on PL class composition. A small increase in SM with age was noted in liver but there were no changes with age in the proportions of CL in either liver or mitochondria. In contrast, individual PL fatty acid compositions showed marked changes in both liver tissue and mitochondria of rainbow trout of between 1- and 4-years of age. Phosphoglycerides, the major PL constituents of cell membranes (primarily PC, PE, PI, PS), and CL showed similar differences among the age groups, with a decrease in PIn, mainly reflecting decreased DHA, particularly in CL, PE and PC. This decrease in PIn correlated with the increase of TBARS for most of the mitochondrial PL, with a major change between 2 and 4 years of age. However, there was no correlation for liver tissue. Most of the PL classes also showed a decrease in total saturated fatty acids and an increase in total monounsaturated fatty acids. The liver tissue and mitochondria of 4 year-old fish had lower DHA contents but similar proportions of total PUFA due to an increase in EPA, 22:5n-3 and, in most of the PL, an increase in n-

6 PUFA. Since DHA is the fatty acid most sensitive to peroxidation and. considering the increase in lipid peroxidation with age, these changes may be indicating oxidation of DHA and the existence of a compensatory mechanism in cell membranes in order to maintain membrane fluidity. In a previous study on the killifish, *Nothobranchius korthausae,* fatty acid composition of undifferentiated, adult and senescent fish were compared (Lucas-Sánchez *et al.* 2011). Analyses of whole animals revealed that DHA increased from undifferentiated to adult fish and then decreased in senescent animals, in which no growth was detected, leading the authors to suggest the changes were related with the aging process. Within his theory of the membrane pacemaker of animal metabolism, Hulbert established possible links between cell membrane composition, metabolic rate and life-span, pointing to membrane composition as the catalyst for the processes involved in cumulative damage to cell molecules and dysfunction during periods of high oxidative stress and aging (Hulbert 2007, 2008). To our knowledge, there are no studies analysing changes in tissue and mitochondrial membrane PL composition with age in any vertebrate, including fish. However, the present data are consistent with these relationships operating in trout liver and mitochondrial membranes. The considerable and rapid increase in body size during the early years of the trout's life-cycle could be one determining factor in the modulation of species life-span, specifically accelerating the aging process. During this rapid growth phase tissue metabolism and antioxidant defence mechanisms could be modified, this fact promoting lipid peroxidation and substantially affecting PL fatty acid composition. Considering the importance of PL fatty acid composition and the role of specific PL, particularly CL, in mitochondrial function and cell viability, these changes could be affecting ETC efficiency, ROS production and signalling systems (Paradies *et al.* 2002), and be mediators of the processes involved in the species response to oxidative stress and damage accumulation rate (aging).

In addition to changes in the phosphoglycerides, SM composition also changed with age. Interestingly, those changes were in generally opposite to those observed in the other PL classes. Mitochondrial membrane SM showed an increase of PIn, with no significant change in DHA content, an increase in total PUFA and saturated fatty acids, and a decline in total monounsaturated fatty acids. Increased EPA and 22:5n-3 with age was also observed, which could be considered markers of older/larger trout in all PL classes of liver mitochondria. Furthermore, decreased percentage of 24:1n-9 with age was

also observed in liver mitochondria. Unlike phosphoglycerides and CL, trends in SM fatty acid compositions were different for liver tissue and mitochondria among the age groups. Total liver showed fewer changes than mitochondria, most of them between 1- and 2-year-old fish with 1-year values being restored in 4-year-old trout. However, mitochondrial SM fatty acids were more unsaturated and therefore more prone to oxidation. This is important since it was suggested that factors that alter SM metabolism, including oxidative and metabolic stress, also increase the risk and progression of age-related diseases (Cutler and Mattson 2001). Therefore, these alterations of SM content and fatty acid composition in trout liver and mitochondria during their four first years of life could also indicate an age-related deterioration which could lead to mitochondrial dysfunction.

In summary, the present study showed an increase in lipid peroxidation in both liver tissue and liver mitochondria during the first four years of life in rainbow trout. These data, along with those showing a decrease in antioxidant defences and increased oxidative damage appear to confirm the existence of high oxidative stress and marked damage to liver membrane lipids. Although there were no major or consistent effects of age on PL class composition, the individual PL class fatty acid compositions were significantly affected, which could considerably alter their properties as the major constituents of cellular membranes including mitochondria. Particularly important were the changes observed in CL and SM fatty acid compositions as these PL have been proposed as mediators in mitochondrial dysfunction and apoptosis as consequences of situations of high oxidative stress and aging.

5. Conclusions

The present study investigated the effects of rapid growth and aging on lipid and fatty acid compositions of liver tissue and mitochondria of a teleost fish species, rainbow trout. There were no previous studies addressing mitochondrial membrane PL compositions during the life-cycle of fish. During the first years of life, liver tissue and mitochondria showed increased lipid peroxidation associated with alterations (damage) to membrane PL that increased with age. These data, along with previous data reporting increasing accumulation of protein oxidation and a decrease in activity of antioxidant systems, point to the existence of oxidative stress associated to rapid growth and

maturation. Following the membrane pacemaker theory of animal metabolism, lipid could be among the first molecules affected by mitochondrial ROS, and lipid peroxidation could be the propagator of oxidative damage reactions. Liver tissue and mitochondrial membranes have different PL compositions reflecting adaptation of membranes to specific functions. Some changes were found in the proportions of PL classes and, especially, in PL fatty acid composition. Since the specific properties of individual PL depend on their fatty acid compositions the observed changes will likely affect membrane structure and function. Mitochondrial-specific CL has been suggested to play a key role as regulator of ETC, and SM appears to be implied in signalling systems mediating cell survival. Further investigation of the mechanisms involved in oxidative stress situations and aging is required and fish can be an important tool in these studies.

Acknowledgements

This project was funded by a Fundación Ramón Areces post-doctoral grant to P.F. Almaida-Pagán.

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485 **Figure legend**

486 Figure 1. TBARS contents (nmol/ g lipid) of liver tissue and liver mitochondria of rainbow trout of
487 three different ages (1-, 2- and 4-year-old). Data expressed as media ± SD (n=3). Letters represent the
488 existence of statistical differences among age groups for each liver tissue and mitochondria ($P< 0.05$,
489 one-way ANOVA). Asterisks denote significant differences between total liver and liver mitochondria
490 ($P< 0.05$, t-student).

Table 1. Fatty acid composition (percentage of total fatty acids) of 1-2 and 4 year-old rainbow trout diets.

Fatty acid	Feeds	
	1-2	4
14:0	6.9	7.6
16:0	19.0	19.0
18:0	3.7	5.4
∑saturated[a]	30.5	33.4
16:1n-7	8.0	8.2
18:1n-7	3.1	3.5
18:1n-9	10.7	8.9
24:1n-9	0.9	0.6
∑monounsaturated[b]	25.8	23.3
18:2n-6	6.6	4.3
20:4n-6	0.9	1.0
∑n-6 PUFA[c]	8.4	6.1
18:3n-3	0.9	1.0
18:4n-3	2.2	2.3
20:4n-3	0.6	0.6
20:5n-3	15.3	16.3
22:5n-3	1.9	2.0
22:6n-3	9.9	9.6
∑n-3 PUFA[d]	30.8	31.9
∑n-3 LC-PUFA	27.7	28.6

LC-PUFA, long-chain PUFA; PUFA, polyunsaturated fatty acids.

[a] Totals include 15:0, 20:0 and 22:0.

[b] Totals include 16:1n-9, 20:1n-9, 20:1n-7, 22:1n-9 and 22:1n-9.

[c] Totals include 18:3n-6, 20:2n-6, 20:3n-6. 22:4n-6 and 22:5n-6.

[d] Totals include 20:3n-3 and 22:4n-3.

Table 2. Biometric data of rainbow trout age groups.

	Age groups		
	1 year (n=21)	2 years (n=12)	4 years (n=3)
Weight (g)	37.9±12.9	115.3±39.6	2986.3±135.9
Length (cm)	14.3±1.9	20.6±2.4	60.0±5.0
K	1.3±0.2	1.3±0.5	1.4±0.3

Data expressed as mean ± SD. n, number of individuals; K, condition factor.

Table 3. Phospholipid content (percentage of total lipid) and phospholipid class composition (percentage of total phospholipids) of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

	Liver tissue			Liver mitochondria		
	1 year	2 years	4 years	1 year	2 years	4 years
∑PL	61.1 ± 4.0^{a*}	66.5 ± 2.6^{a,b*}	69.5 ± 3.0^{c}	82.9 ± 1.1^{c}	78.5 ± 2.3^{b}	73.3 ± 3.3^{a}
PC	38.6 ± 2.6*	38.3 ± 1.4	39.9 ± 1.2	33.4 ± 0.7^{a}	37.9 ± 0.6^{b}	35.3 ± 2.7^{a,b}
PE	33.6 ± 0.7^{c*}	26.8 ± 1.0^{a}	29.5 ± 1.3^{b*}	31.6 ± 1.2^{b}	27.9 ± 1.6^{a}	32.9 ± 0.4^{b}
PI	9.3 ± 1.0^{a}	11.2 ± 0.7^{b*}	9.7 ± 0.7^{a,b}	9.6 ± 0.6	9.0 ± 0.4	9.6 ± 0.1
CL	4.6 ± 1.3*	5.4 ± 0.4*	4.8 ± 1.1*	9.2 ± 0.8	9.4 ± 1.3	8.3 ± 2.1
PS	4.8 ± 0.8^{a}	8.0 ± 0.5^{b*}	7.1 ± 0.8^{b*}	5.5 ± 0.8	5.1 ± 0.4	5.1 ± 0.3
SM	3.2 ± 1.0^{a}	5.2 ± 0.4^{b*}	5.4 ± 0.8^{b*}	3.6 ± 0.7	4.1 ± 0.6	3.8 ± 0.7

Results are means ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). Asterisks denote statistical differences between liver tissue and mitochondria from liver when compared using a t-test (P<0.05). PL, phospholipid; CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, total polar lipids; PS, phosphatidylserine; SM, sphingomyelin.

Table 4. Fatty acid composition (percentage of total fatty acids) of phosphatidylcholine of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

	Liver tissue			Liver mitochondria		
Fatty acid	1 year	2 years	4 years	1 year	2 years	4 y
14:0	2.8 ± 0.2[b]	2.9 ± 0.4[b]	1.7 ± 0.4[a]	3.1 ± 0.2[c]	2.9 ± 0.1[b]	1.8
16:0	24.0 ± 1.0[a,c]	24.9 ± 1.2[b]	22.5 ± 0.4[a]	25.1 ± 0.5[b]	25.9 ± 0.9[b]	21.
18:0	2.1 ± 0.6[a]	3.5 ± 0.3[b]	6.4 ± 1.0[c]	2.3 ± 0.0[a]	3.3 ± 0.4[b]	6.
∑saturated[a]	29.4 ± 0.6[a]	32.3 ± 0.7[b]	31.9 ± 0.5[b]	31.1 ± 0.6[a]	32.6 ± 1.2[b]	30.
16:1n-7	3.9 ± 0.1[b]	3.4 ± 0.3[b]	2.2 ± 0.4[a]	5.1 ± 0.3[c]	3.2 ± 0.2[b]	1.9
18:1n-7	0.7 ± 0.7[a]	1.8 ± 0.4[a,b]	5.0 ± 0.8[b]	1.8 ± 0.1[b]	1.5 ± 0.1[a]	5.4
18:1n-9	6.5 ± 0.9	5.3 ± 0.9	6.8 ± 0.5	5.0 ± 0.3[a]	5.5 ± 0.5[a]	6.2
20:1n-9	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0[a]	0.5 ± 0.1[b]	0.9
24:1n-9	0.8 ± 0.2[b]	0.2 ± 0.1[a]	0.3 ± 0.1[a]	2.1 ± 0.7[b]	0.7 ± 0.7[a]	0.4
∑monounsaturated[b]	11.8 ± 0.9[a]	10.8 ± 1.6[a]	14.5 ± 0.4[b]	14.1 ± 0.9[b]	11.5 ± 1.1[a]	14.
18:2n-6	1.1 ± 0.0[a]	1.0 ± 0.1[a]	1.8 ± 0.5[b]	1.2 ± 0.0[a]	1.0 ± 0.0[a]	1.9
20:4n-6	1.3 ± 0.1[a]	1.9 ± 0.1[b]	1.8 ± 0.3[b]	1.3 ± 0.1[a]	1.9 ± 0.1[b]	1.8
∑n-6 PUFA[c]	3.2 ± 0.1[a]	4.4 ± 0.4[b]	4.4 ± 0.7[b]	3.3 ± 0.2[a]	4.5 ± 0.2[b]	5.0
20:4n-3	0.4 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.0[a]	0.3 ± 0.1[a]	0.6
20:5n-3	9.2 ± 0.9[a]	10.2 ± 0.5[a]	14.2 ± 2.4[b]	8.6 ± 0.4[a]	10.1 ± 1.0[b]	14.
22:5n-3	1.9 ± 0.1[a]	2.1 ± 0.2[a]	5.0 ± 0.7[b]	1.7 ± 0.1[a]	2.4 ± 0.2[b]	5.1
22:6n-3	42.9 ± 0.4[c]	38.8 ± 1.8[b]	28.2 ± 2.5[a]	40.1 ± 0.2[c]	37.9 ± 1.2[b]	28.
∑n-3 PUFA[d]	54.9 ± 0.5[c]	51.9 ± 1.4[b]	48.6 ± 0.8[a]	51.0 ± 0.7	51.0 ± 2.4	49
∑PUFA	58.7 ± 0.4[c]	56.9 ± 1.4[b]	53.6 ± 0.3[a]	54.8 ± 0.8	53.9 ± 2.1	54
∑n-3 LC-PUFA	54.4 ± 0.6[b]	51.3 ± 1.5[b]	48.0 ± 0.8[a]	50.7 ± 0.7	50.7 ± 0.4	49
n-3/n-6	17.2 ± 0.5[b]	11.8 ± 1.1[a]	11.2 ± 2.0[a]	15.5 ± 0.5[c]	11.5 ± 1.0[b]	10.
PIn	423.1 ± 1.5[c]	402.8 ± 12.6[b]	356.9 ± 7.9[a]	394.9 ± 4.9[b]	395.4 ± 16.2[b]	364

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids.

[a] Totals include 15:0, 20:0 and 22:0 present up to 0.2%.

[b] Totals include 16:1n-9, 20:1n-7 and 22:1n-9 present up to 0.1%.

[c] Totals include 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 present up to 0.7%.

[d] Totals include 18:3n-3, 18:4n-3, 20:3n-3 and 22:4n-3 present up to 0.3%.

Table 5. Fatty acid composition (percentage of total fatty acids) of phosphatidylethanolamine of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

	Liver tissue			Liver mitochondria		
Fatty acid	1 year	2 years	4 years	1 year	2 years	4 years
14:0	0.7 ± 0.5	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.2	0.2 ± 0.0
16:0	10.4 ± 1.4	10.4 ± 1.3	8.8 ± 0.6	11.7 ± 0.4^{b}	14.0 ± 0.7^{c}	9.7 ± 0.6
18:0	6.8 ± 1.1^{a}	9.1 ± 0.8^{b}	6.6 ± 1.3^{a}	6.4 ± 0.7^{a}	8.3 ± 0.5^{b}	6.5 ± 1.1
∑saturated^{a}	18.8 ± 3.3	20.5 ± 2.0	15.9 ± 0.8	18.7 ± 0.9^{b}	22.9 ± 0.9^{c}	16.4 ± 1.
16:1n-7	3.0 ± 0.5^{b}	1.7 ± 0.4^{a}	1.3 ± 0.2^{a}	2.3 ± 0.2^{b}	2.2 ± 0.5^{b}	0.9 ± 0.1
18:1n-7	4.2 ± 0.8^{a}	3.5 ± 0.4^{a}	8.8 ± 1.9^{b}	6.8 ± 0.4^{b}	5.3 ± 0.2^{a}	12.6 ± 0.
18:1n-9	11.9 ± 0.6^{b}	9.4 ± 0.6^{a}	11.2 ± 2.2^{a,b}	8.7 ± 1.0^{b}	7.5 ± 0.7^{a}	8.1 ± 0.5
20:1n-9	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.8 ± 0.0^{a}	1.5 ± 0.1^{b}	1.9 ± 0.3
∑monounsaturated^{b}	19.7 ± 0.3^{b}	15.4 ± 1.1^{a}	21.9 ± 1.3^{c}	19.0 ± 1.1^{b}	17.1 ± 1.0^{a}	23.7 ± 1.
18:2n-6	4.3 ± 0.2^{c}	1.7 ± 0.1^{a}	3.2 ± 0.4^{b}	4.3 ± 0.2^{c}	2.0 ± 0.2^{a}	3.8 ± 0.6
18:3n-6	0.3±0.3^{a}	0.4±0.1^{a}	0.7±0.0^{b}	0.7±0.0^{a}	0.5±0.1^{a}	1.0±0.1
20:2n-6	0.3±0.3	0.8±0.2	0.7±0.2	0.6±0.1	0.9±0.2	0.8±0.1
20:3n-6	0.4±0.1	0.7±0.2	0.9±0.4	0.4±0.0^{b}	0.4±0.0^{b}	0.2±0.0
20:4n-6	1.9 ± 0.4^{a}	2.4 ± 0.2^{a}	5.5 ± 0.6^{b}	2.2 ± 0.2^{a}	2.7 ± 0.3^{a}	5.8 ± 0.9
∑n-6 PUFA^{c}	7.9 ± 1.0^{a}	7.2 ± 0.4^{a}	11.3 ± 1.0^{b}	8.6 ± 0.3^{b}	7.2 ± 0.6^{a}	11.3 ± 1.
18:3n-3	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.
20:4n-3	n.d.	n.d.	0.5 ± 0.2	0.7 ± 0.1c	0.4 ± 0.1a	0.6 ± 0.0
20:5n-3	7.4 ± 0.3^{a}	6.0 ± 0.8^{a}	20.5 ± 2.6^{b}	6.7 ± 0.5^{a}	5.8 ± 0.2^{a}	19.0 ± 2.
22:5n-3	1.8 ± 0.3^{a}	1.7 ± 0.3^{a}	4.1 ± 0.5^{b}	1.4 ± 0.0^{a}	1.6 ± 0.2^{a}	3.5 ± 0.5
22:6n-3	43.1 ± 2.2^{b}	47.7 ± 1.3^{c}	24.4 ± 2.6^{a}	43.9 ± 0.8^{b}	43.8 ± 1.1^{b}	24.7 ± 2.
∑n-3 PUFA^{d}	52.9 ± 2.2^{a,b}	55.9 ± 1.2^{b}	50.2 ± 0.9^{a}	53.2 ± 0.6^{c}	52.1 ± 1.4^{b}	48.3 ± 0.
∑PUFA	61.4 ± 3.0	64.0 ± 1.3	62.1 ± 0.7	62.4 ± 0.5	60.1 ± 1.5	59.9 ± 1.
∑n-3 LC-PUFA	52.4 ± 2.2^{a}	55.5 ± 1.2^{b}	49.6 ± 0.9^{a}	52.9 ± 0.6^{c}	51.7 ± 1.4^{b}	48.0 ± 0.
n-3/n-6	6.8 ± 0.7^{b}	7.8 ± 0.5^{b}	4.5 ± 0.5^{a}	6.2 ± 0.3^{b}	7.3 ± 0.7^{c}	4.3 ± 0.6
PIn	452.1 ± 63.2	452.4 ± 9.9	378.6 ± 7.5	424.2 ± 4.1^{b}	419.5 ± 10.8^{b}	367.2 ± 3

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids, n.d., non-detectable.

[a] Totals include 15:0, 20:0 and 22:0 present up to 0.5%.

[b] Totals include 16:1n-9, 20:1n-7, 22:1n-9 and 24:1n-9 present up to 0.5%.

[c] Totals include 22:4n-6 and 22:5n-6 present up to 0.7%.

[d] Totals include 18:4n-3, 20:3n-3 and 22:4n-3 present up to 0.2%.

Table 6. Fatty acid composition (percentage of total fatty acids) of cardiolipin of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

Fatty acid	Liver tissue			Liver mitochondria		
	1 year	2 years	4 years	1 year	2 years	4 years
14:0	1.8 ± 0.3^{a,b}	2.7 ± 0.8^{b}	1.2 ± 0.1^{a}	2.0 ± 0.1^{b}	2.9 ± 0.3^{c}	1.1 ± 0.2^{a}
16:0	20.0 ± 1.2^{b}	14.0 ± 2.4^{a}	20.3 ± 2.0^{b}	20.9 ± 0.7^{b}	4.8 ± 0.5^{a}	21.8 ± 0.9^{c}
18:0	4.4 ± 0.7^{b}	2.0 ± 0.6^{a}	4.1 ± 1.2^{b}	3.3 ± 0.8^{b}	1.6 ± 0.2^{a}	4.0 ± 0.3^{c}
∑saturated[a]	27.4 ± 1.5^{b}	19.6 ± 1.4^{a}	27.1 ± 3.8^{b}	26.7 ± 1.3^{b}	9.7 ± 0.7^{a}	27.3 ± 0.8^{b}
16:1n-7	5.7 ± 0.4^{a}	9.0 ± 2.2^{b}	3.4 ± 1.4^{a}	5.9 ± 0.2^{b}	12.1 ± 1.0^{c}	1.7 ± 0.7^{a}
18:1n-7	2.9 ± 0.4^{a}	1.6 ± 0.1^{a}	6.5 ± 2.6^{b}	5.1 ± 0.5^{b}	2.2 ± 0.3^{a}	9.2 ± 1.1^{c}
18:1n-9	7.2 ± 0.5	7.2 ± 2.5	9.2 ± 0.8	5.4 ± 0.2	5.6 ± 0.2	5.8 ± 0.9
20:1n-9	0.1±0.0	0.2±0.0	0.1±0.0	0.4±0.2^{a}	0.6±0.1^{a}	0.9±0.2^{b}
24:1n-9	1.2 ± 0.4	1.1 ± 0.5	1.4 ± 0.3	0.9 ± 0.5^{b}	2.1 ± 0.7^{c}	0.2 ± 0.3^{a}
∑monounsaturated[b]	17.3 ± 0.7	19.2 ± 5.2	21.0 ± 4.2	17.6 ± 0.5^{a}	22.7 ± 0.7^{b}	18.0 ± 2.8^{a}
18:2n-6	4.9 ± 0.5^{a}	3.9 ± 0.3^{a}	6.5 ± 0.8^{b}	5.1 ± 0.3^{a}	6.2 ± 0.2^{b}	6.0 ± 0.9^{b}
20:2n-6	1.4±0.3^{a}	0.9±0.4^{a}	2.1±0.4^{b}	1.6±0.2^{a}	1.3±0.3^{a}	2.5±0.6^{b}
20:3n-6	0.6±0.3	0.7±0.1	1.2±0.5	0.4±0.0^{a}	1.0±0.1^{b}	0.4±0.1^{a}
20:4n-6	1.6 ± 0.4	3.0 ± 0.6	3.6 ± 1.4	1.4 ± 0.6^{a}	1.1 ± 0.1^{a}	4.0 ± 0.9^{b}
∑n-6 PUFA[c]	9.0 ± 0.4^{a}	9.5 ± 1.1^{a}	13.9 ± 2.2^{b}	8.9 ± 0.7^{a}	10.4 ± 0.3^{b}	13.6 ± 2.1^{c}
18:3n-3	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}	0.8 ± 0.1^{b}	0.9 ± 0.0^{a}	1.4 ± 0.0^{c}	1.0 ± 0.1^{b}
18:4n-3	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.3 ± 0.2^{b}	0.5 ± 0.1^{b}	0.1 ± 0.1^{a}
20:4n-3	0.6 ± 0.2	1.3 ± 0.5	1.2 ± 0.5	0.7 ± 0.0^{a}	1.5 ± 0.3^{b}	1.8 ± 0.5^{b}
20:5n-3	1.4 ± 0.2^{a}	3.2 ± 1.2^{b}	3.9 ± 1.7^{a,b}	1.3 ± 0.1^{a}	2.2 ± 0.8^{a}	5.9 ± 3.5^{b}
22:5n-3	3.6 ± 0.7^{a}	3.8 ± 0.9^{a}	6.4 ± 1.0^{b}	3.5 ± 0.3^{a}	4.9 ± 0.3^{a}	6.5 ± 0.6^{b}
22:6n-3	38.6 ± 1.4^{b}	41.0 ± 2.7^{b}	24.4 ± 2.6^{a}	38.9 ± 1.2^{b}	44.9 ± 1.3^{c}	24.6 ± 3.4^{a}
∑n-3 PUFA[d]	45.3 ± 1.5^{b}	50.1 ± 5.3^{b}	37.4 ± 1.3^{a}	45.7 ± 1.5^{b}	55.6 ± 1.1^{c}	40.4 ± 4.3^{a}
∑PUFA	55.3 ± 1.5^{a}	61.1 ± 3.8^{b}	51.9 ± 1.2^{a}	55.7 ± 1.0^{a}	67.6 ± 1.0^{b}	54.7 ± 2.3^{a}
∑n-3 LC-PUFA	44.4 ± 1.3^{b}	49.5 ± 5.3^{b}	36.4 ± 1.3^{a}	44.6 ± 1.7^{b}	53.8 ± 1.1^{c}	39.4 ± 4.5^{a}
n-3/n-6	5.0 ± 0.3^{b}	5.4 ± 1.1^{b}	2.8 ± 0.6^{a}	5.2 ± 0.6^{b}	5.3 ± 0.2^{b}	3.0 ± 0.7^{a}
PIn	361.8 ± 11.7^{b}	403.1 ± 33.5^{b}	293.7 ± 12.9^{a}	363.4 ± 9.4^{b}	432.5 ± 7.7^{c}	310.8 ± 27.9^{a}

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids.

[a] Totals include 15:0, 20:0 and 22:0 present up to 0.7%.
[b] Totals include 16:1n-9, 20:1n-7 and 22:1n-9 present up to 0.1%.
[c] Totals include 18:3n-6, 22:4n-6 and 22:5n-6 present up to 0.7%.
[d] Totals include 20:3n-3 and 22:4n-3 present up to 0.5%.

Table 7. Fatty acid composition (percentage of total fatty acids) of phosphatidylinositol of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

Fatty acid	Liver tissue			Liver mitochondria	
	1 year	2 years	4 years	1 year	2 years
14:0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	0.4 ± 0.0
16:0	6.3 ± 0.3[a]	7.5 ± 0.7[b]	9.8 ± 0.6[c]	7.1 ± 0.6[a]	8.1 ± 0.8b
18:0	34.4 ± 0.5[b]	32.8 ± 0.9[b]	24.4 ± 2.7[a]	35.7 ± 0.3[c]	34.0 ± 1.8[b]
∑saturated[a]	42.3 ± 0.8[b]	41.7 ± 1.2[b]	35.6 ± 2.1[a]	43.7 ± 0.6[b]	42.8 ± 1.1[b]
16:1n-7	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.5	0.7 ± 0.1
18:1n-7	1.1 ± 0.2[a]	0.9 ± 0.2[a]	2.7 ± 0.6[b]	0.9 ± 0.8[a]	1.1 ± 0.4[a]
18:1n-9	5.0 ± 0.3[a]	4.9 ± 0.5[a]	7.5 ± 0.7[b]	2.7 ± 1.3[a]	4.4 ± 0.5[b]
20:1n-9	0.1±0.1	0.1±0.0	0.1±0.1	0.4±0.0[a]	0.5±0.1[a]
24:1n-9	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.4	0.3 ± 0.0
∑monounsaturated[b]	7.3 ± 0.7[a]	6.9 ± 0.8[a]	11.4 ± 1.5[b]	5.5 ± 1.7[a]	7.2 ± 1.0[b]
18:2n-6	0.5 ± 0.1[a]	0.4 ± 0.0[a]	0.9 ± 0.3[b]	0.6 ± 0.1[b]	0.2 ± 0.1[a]
20:4n-6	37.3 ± 1.2	35.2 ± 2.6	36.8 ± 2.4	37.2 ± 1.3[b]	33.7 ± 1.7[a]
∑n-6 PUFA[c]	38.2 ± 1.1	36.2 ± 2.5	38.2 ± 1.8	38.6 ± 1.2[b]	35.1 ± 1.3[a]
20:5n-3	2.6 ± 0.8	1.7 ± 0.5	3.0 ± 0.9	2.3 ± 0.2[b]	1.6 ± 0.6[a]
22:5n-3	0.9 ± 0.2[a]	1.2 ± 0.2[a]	2.4 ± 0.3[b]	0.9 ± 0.1[a]	1.2 ± 0.2[b]
22:6n-3	8.0 ± 1.9[a]	11.2 ± 1.7[b]	7.7 ± 1.0[a]	7.9 ± 0.2[a]	10.7 ± 1.6[b]
∑n-3 PUFA[d]	11.7 ± 1.7	14.2 ± 2.2	14.0 ± 2.0	11.6 ± 0.4[a]	13.6 ± 1.5[b]
∑PUFA	50.8 ± 0.7[a]	51.4 ± 0.8[a]	53.0 ± 0.7[b]	50.8 ± 1.3	50.1 ± 0.3
∑n-3 LC-PUFA	11.6 ± 1.7	14.1 ± 2.2	13.7 ± 2.2	11.3 ± 0.3[a]	13.5 ± 1.6[b]
PIn	270.3 ± 51.5	284.9 ± 49.0	247.6 ± 6.2	237.5 ± 5.3	243.7 ± 6.5

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA ($P<0.05$). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids.

[a] Totals include 15:0, 20:0 and 22:0 present up to 0.8%.

[b] Totals include 16:1n-9, 20:1n-7 and 22:1n-9 present up to 0.1%.

[c] Totals include 18:3n-6, 20:2n-6, 22:3n-6, 22:4n-6 and 22:5n-6 present up to 0.4%.

[d] Totals include 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3 and 22:4n-3 present up to 0.3%.

Table 8. Fatty acid composition (percentage of total fatty acids) of phosphatidylserine of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

	Liver tissue			Liver mitochondria		
Fatty acid	1 year	2 years	4 years	1 year	2 years	4
14:0	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.8 ± 0.3	
15:0	0.5±0.2	0.4±0.2	1.0±0.2	0.3±0.0	0.3±0.0	
16:0	14.6 ± 0.9	15.1 ± 1.3	15.3 ± 1.3	15.5 ± 0.6	16.3 ± 1.7	
18:0	22.3 ± 0.3[b]	22.8 ± 1.6[b]	15.5 ± 1.7[a]	21.1 ± 1.7[b]	21.0 ± 2.8[b]	
∑saturated[a]	38.4 ± 0.4[b]	39.8 ± 2.3[b]	32.7 ± 2.7[a]	38.2 ± 1.0[b]	39.0 ± 1.1[b]	
16:1n-7	0.8 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	1.2 ± 0.5	1.1 ± 0.1	
18:1n-7	2.4 ± 0.1[a]	2.0 ± 0.1[a]	5.9 ± 1.5[b]	2.5 ± 0.5[a]	2.1 ± 0.7[a]	
18:1n-9	2.7 ± 0.4[a]	3.1 ± 0.5[a]	5.4 ± 0.4[c]	3.5 ± 0.3[a]	3.7 ± 0.7[a]	
20:1n-9	0.6±0.1	0.5±0.1	0.8±0.7	0.5±0.0[a]	0.6±0.0[a]	
24:1n-9	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.5 ± 0.6b	0.9 ± 0.3a	
∑monounsaturated[b]	7.5 ± 0.4[a]	7.8 ± 0.6[a]	13.9 ± 2.1[b]	9.9 ± 1.7[a]	9.0 ± 0.5[a]	
18:2n-6	0.8 ± 02	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2[b]	0.4 ± 0.1[a]	
20:4n-6	1.0 ± 0.8	0.8 ± 0.1	1.7 ± 0.4	0.6 ± 0.1[a]	1.0 ± 0.3[b]	
22:5n-6	0.8±0.1	1.2±0.3	n.d.	0.7±0.1[b]	0.9±0.1[b]	
∑n-6 PUFA[c]	3.3 ± 1.0	3.5 ± 0.6	3.6 ± 0.6	2.6 ± 0.2[a]	2.8 ± 0.4[a]	
20:5n-3	1.5 ± 0.3[a]	1.9 ± 0.3[a]	5.0 ± 0.8[b]	1.4 ± 0.3[a]	2.4 ± 1.2[b]	
22:5n-3	1.5 ± 0.1[a]	1.6 ± 0.1[a]	7.5 ± 0.8[b]	1.4 ± 0.1[a]	1.8 ± 0.3[a]	
22:6n-3	46.1 ± 2.0[b]	43.5 ± 1.5[b]	35.5 ± 1.7[a]	44.7 ± 1.5[b]	43.1 ± 1.2[b]	
∑n-3 PUFA[d]	49.4 ± 1.8	47.4 ± 1.7	48.6 ± 1.6	48.1 ± 1.5	47.5 ± 1.6	
∑PUFA	54.2 ± 0.7	52.4 ± 2.0	53.3 ± 1.6	51.9 ± 1.1	52.0 ± 1.5	
∑n-3 LC-PUFA	49.1 ± 1.8	47.9 ± 0.6	48.2 ± 1.6	47.7 ± 1.6	47.3 ± 1.7	
n-3/n-6	15.9 ± 4.8	13.9 ± 2.8	13.8 ± 2.2	18.4 ± 0.7[b]	16.9 ± 2.7[b]	
PIn	401.6 ± 11.3	386.1 ± 13.8	373.3 ± 11.7	388.1 ± 11.9[b]	384.9 ± 11.4[b]	

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids; n.d., non-detectable.

[a] Totals include 20:0 and 22:0 present up to 0.7%.

[b] Totals include 16:1n-9, 20:1n-7 and 22:1n-9 present up to 0.5%.

[c] Totals include 18:3n-6, 20:2n-6, 22:3n-6 and 22:4n-6 present up to 0.4%.

[d] Totals include 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3 and 22:4n-3 present up to 0.2%.

Table 9. Fatty acid composition (percentage of total fatty acids) of sphingomyelin of liver and mitochondria isolated from liver of 1-, 2- and 4-year-old rainbow trout.

	Liver tissue			Liver mitochondria		
Fatty acid	1 year	2 years	4 years	1 year	2 years	4 ye
14:0	3.7 ± 0.8[a]	3.7 ± 0.0[a]	8.2 ± 0.3[b]	3.8 ± 0.6	3.9 ± 0.3	8.
15:0	1.1±0.2	0.7±0.3	1.8±0.8	0.8±0.2	0.7±0.1	0
16:0	16.1 ± 3.0[a]	24.9 ± 3.7[b]	13.9 ± 0.3[a,*]	19.7 ± 1.6	20.4 ± 1.4	21
18:0	4.9 ± 0.6	5.9 ± 0.3	4.7 ± 1.9	5.2 ± 0.3[a]	6.3 ± 0.8[b]	6.
∑saturated[a]	26.1 ± 3.9[a]	36.1 ± 3.3[b]	29.5 ± 2.2[a,b,*]	29.4 ± 1.2[a]	31.9 ± 1.7[b]	36
16:1n-7	3.5 ± 0.3	4.3 ± 1.2	2.7 ± 0.2	3.2 ± 0.5[a,b]	2.3 ± 1.0[a]	3.
18:1n-7	0.2 ± 0.1	0.1 ± 0.1	n.d.	1.2 ± 0.0[a]	1.4 ± 0.5[b]	2.
18:1n-9	9.4 ± 3.2[a]	18.3 ± 2.9[b]	10.1 ± 1.2[a]	8.2 ± 0.4[a]	6.6 ± 2.0[a]	8.
20:1n-9	n.d.	0.9±0.2	0.4±0.3	0.2±0.2	0.5±0.2	0
24:1n-9	42.8 ± 3.5[b]	21.8 ± 9.1[a,*]	43.3 ± 4.2[b,*]	40.3 ± 10.0[b]	36.8 ± 3.2[b]	22
∑monounsaturated[b]	57.2 ± 1.7	45.9 ± 5.4	57.5 ± 5.9[*]	53.0 ± 5.8[b]	48.2 ± 0.7[b]	37
18:2n-6	1.0 ± 0.2[a]	1.6 ± 0.2[b]	1.1 ± 0.1[a]	0.7 ± 0.2[a]	1.0 ± 0.4[b]	1.
20:4n-6	n.d.	0.9 ± 0.1[a]	0.2 ± 0.0[b]	0.4 ± 0.4	0.5 ± 0.1	0.
∑n-6 PUFA[c]	3.8 ± 2.0	5.1 ± 0.7[*]	2.8 ± 0.5	1.5 ± 1.1	2.3 ± 0.3	2.
18:4n-3	0.3 ± 0.1[b]	0.1 ± 0.0[a]	0.4 ± 0.0[b]	1.0 ± 0.4	0.6 ± 0.1	0.
20:5n-3	2.0 ± 0.5	2.0 ± 0.4	2.3 ± 0.7[*]	1.9 ± 0.6[a]	2.7 ± 0.8[a]	4.
22:5n-3	0.4 ± 0.1	1.2 ± 0.5	1.0 ± 0.3[*]	0.3 ± 0.5[a]	0.9 ± 0.2[a]	1.
22:6n-3	8.5 ± 2.3	8.4 ± 1.1	4.6 ± 2.0[*]	11.2 ± 2.7	11.9 ± 0.3	13
∑n-3 PUFA[d]	11.5 ± 3.0	12.2 ± 1.6[*]	8.4 ± 2.9[*]	14.4 ± 3.7[a]	16.2 ± 0.9[a]	21
∑PUFA	16.6 ± 5.0	18.0 ± 2.1	13.0 ± 3.7[*]	17.6 ± 4.7[a]	19.9 ± 1.0[a]	25
∑n-3 LC-PUFA	11.1 ± 2.8	11.9 ± 1.7[*]	8.0 ± 2.9[*]	13.4 ± 3.9 [a]	15.6 ± 0.9[a,b]	20
PIn	97.1 ± 27.2	103.7 ± 12.3[*]	69.2 ± 23.4[*]	116.8 ± 30.6[a]	130.6 ± 5.8[a,b]	163

Data expressed as mean ± S.D. (n = 3). Different superscript letters within a row and for each sample type (liver or mitochondria) represent significant differences between age groups as determined by one-way ANOVA (P<0.05). Asterisks denote statistical differences between liver tissue and mitochondria from liver when compared using a t-test (P<0.05). LC-PUFA, long-chain polyunsaturated fatty acids; PIn, peroxidation index; PUFA, polyunsaturated fatty acids; n.d., non-detectable.

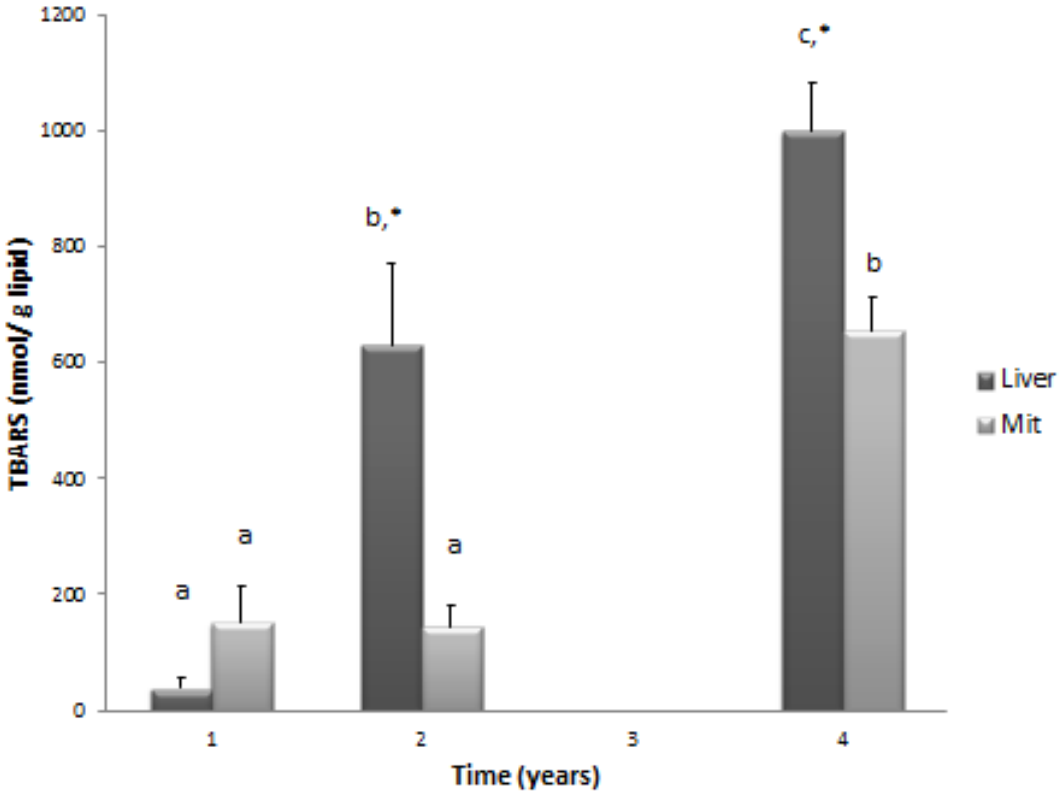
[a] Totals include 20:0 and 22:0 present up to 0.6%.

[b] Totals include 16:1n-9, 20:1n-7 and 22:1n-9 present up to 0.6%.

[c] Totals include 18:3n-6, 20:2n-6, 22:3n-6, 22:4n-6 and 22:5n-6 present up to 0.7%.

[d] Totals include 18:3n-3, 20:3n-3, 20:4n-3 and 22:4n-3 present up to 0.2%.

Figure 1.


1200
1000
800
600
400
200
0
TBARS (nmol/ g lipid)
a
a
b,*
a
c,*
b
1
2
3
4
Time (years)
Liver
Mit