# Effect of Starvation on Lipid Metabolism and Stability of DHA Content of Lipids in Horse Mackerel *(Trachurus japonicus)* Tissues

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**ABSTRACT:** For the purpose of characterizing the effect of starvation on 22:6n-3 (DHA) content in marine fish tissues, horse mackerel *(Trachurus japonicus)* were reared in a tank containing filtered, sterilized seawater under nonfeeding conditions for 107 d (survival rate of the fish was 96.5%). The crude total lipids (TL) of ordinary dorsal muscle, dorsal skin, and viscera of the starved individuals were separated into classes on silicic acid columns, and the constituents of the TL were quantified by gravimetric recovery from column chromatography. The TL, initially >85% TAG in dorsal muscle, and even more in skin lipids, decreased dramatically within the first 44 d of starvation, and then decreased more gradually during the remainder of the test period, whereas the visceral TL decreased more slowly. The percentages of both saturated and monoenoic FA in the muscle TL also decreased somewhat, but those of DHA increased significantly in muscle during the test periods. Decreases in PE and PC initially were much smaller than TAG, but DHA levels remained high in both PE and PC. These findings indicate that all of the FA in the depot lipids of horse mackerel tissues are easily metabolized for energy production during starvation, but DHA in muscle lipids of the starved fish was maintained at a consistently high level, indicating that starvation did not affect DHA stability in phospholipids. The findings suggest that preservation of DHA in cell membrane lipid PE and PC is necessary for self-protection functions in starving fish.

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Lipids play important roles in energy production processes in animal tissues and as a reserve for EFA. Our previous report (1) considered the FA compositions and seasonal variations of saturated and monoenoic FA levels in the tissues of the horse mackerel *(Trachurus japonicus)* from the East China Sea. We noted the occurrence of consistently high levels of PUFA, which were dominated throughout the year by DHA.

Starvation represents an extreme feeding regime that can provide a useful model for the energy budget in standard feeding experiments. Some reports (2–6) suggest that starvation may to some extent be beneficial for human nutrition because it produces a high DHA concentration in the edible tissues of fatty fish. Because a high intake of DHA supports the human body, information on variations in the levels of DHA in fish tissues consumed by people is of dietary significance for human health (7–9).

Considerable attention has been paid to the effects on metabolism, chemical composition, and physiological and microanatomical features of freshwater fish that result from starvation or periods of low energy intake (2–6,10–15). There is very little information, however, on the effect of starvation on the chemical components and physiology of marine fish (14,15), and in particular on the effect of starvation on lipid metabolism.

The present study was undertaken to elucidate the effect of starvation on lipid metabolism, with special attention on DHA stability, in the horse mackerel, an economically important marine fish. The basic purpose of the present study is to provide information on lipid metabolism in starved fish *in vivo* and to elucidate the stability and enrichment of DHA in this system. The potential utility of starved pelagic fatty fish in special formulations of value-added products relating to DHA is considered.

## MATERIALS AND METHODS

*Fish.* Horse mackerel (body weight: 143.4 ± 6.7 g), *T. japonicus,* were caught by fishing with small- to medium-sized purse seines in offshore (10 km from the coast) surface waters near Nagasaki, Japan, and then transported to our laboratory in live condition.

*Fish preservation.* All fish were kept alive for starvation in an elliptical (11 × 7 × 1 m; major axis × minor axis × depth) indoor aquarium (50-ton capacity) at a stocking density of 4 fish/ton of filtered, sterilized seawater. The seawater was continuously refreshed at the rate of 50 ton/d. Water temperature variations (21.7–27.2°C) were measured daily. Salinity (29.00–35.40‰), dissolved oxygen (5.20–7.00 ppm), and pH (7.41–8.42) of the water were measured at 10-d intervals.

*Preparation of sample for lipid analyses.* The fork length (length between mouth and caudal furca) and body weight of the fish were measured. Ordinary dorsal muscles, dorsal skins (including the panniculus and a small amount of muscle), and viscera of the fish were then cut out using a clean scalpel for each tissue.

*Lipid extraction and analyses of lipid classes.* Each tissue sample was minced and homogenized with a mixture of chloroform/methanol (2:1, vol/vol). A portion of homogenized sam-

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Abbreviations: PL, phospholipid; SE, steryl esters; ST, sterols; TL, crude total lipids.

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ple was extracted according to the procedure of Folch *et al.* (16). The crude total lipids (TL) were separated into classes on silicic acid chromatographic columns, and the constituent lipids were quantified by gravimetric recovery of column fractions. The first eluent (dichloromethane/*n*-hexane, 2:3, vol/vol) was used to collect the fraction containing steryl esters (SE). The second eluent (dichloromethane) removed the TAG fraction. This was followed with dichloromethane/ether (9:1, vol/vol), eluting sterols (ST); dichloromethane/methanol (10:1, vol/vol), eluting FFA; dichloromethane/methanol (1:1, vol/vol), eluting PE; and dichloromethane/methanol (1:20, vol/vol), eluting PC. Individual lipids within each lipid class were identified by comparison with standard samples by using TLC (thickness: 0.25 mm for analysis; Kieselgel 60, Merck, Darmstadt, Germany). All sample lipids were dried under nitrogen at room temperature and stored at –70°C in a small amount of dichloromethane.

*Preparation of methyl esters and performance of GLC analysis.* TL and individual lipid components were converted into FAME by direct transesterification with boiling methanol containing a catalytic amount of concentrated sulfuric acid under reflux for 1.5 h. The FAME obtained were separated from other by-products by column chromatography using silica gel (Silica gel 60, 0.063–0.200 mm; Merck) and elution with dichloromethane. Analyses of FAME were performed on a gas chromatograph (GC-17A; Shimadzu Seisakusho Co., Ltd., Kyoto, Japan) equipped with a capillary column (Omegawax-250, 30 m × 0.25 mm i.d., 0.25 μm film thickness; split ratio, 25:1; Supelco Japan Co., Ltd., Tokyo, Japan). The temperatures of the injector, column, and detector were maintained at 290, 205, and 290°C, respectively. Helium was used as the carrier gas at a constant inlet rate of 17 mL/min.

*Statistical analysis.* Regression analysis was conducted using Statview (Version 5.0, SAS Institute Inc., Cary, NC). Slopes of the regression lines (decreasing speed of chemical components) were compared by analysis of covariance (ANCOVA).

## RESULTS AND DISCUSSION

*Variations in TL of organs.* Body size, condition factor, and TL contents of muscle, skin, and viscera, as determined at different intervals of starvation, are presented in Table 1. In general, the TL content in each tissue decreased during starvation. The levels of TL in both skin and muscle decreased more rapidly than those in the viscera in the initial 44 d. The average lipid contents fell from 6.8 to 2.3% in muscle ($Y = -0.09X + 6.18$, $r = -0.60$, $P < 0.05$, $X$: days, $Y$: lipid contents), and from 32.7 to 12.2% in skin ($Y = -0.43X + 29.72$, $r = -0.72$, $P < 0.001$). TL levels fell further in both the muscle and skin, respectively, to 2.2 ± 0.2% and 9.2 ± 0.8% at 107 d. On the other hand, the visceral TL hardly changed (56.0 to 51.4%) within the initial 44 d of starvation, and slowly decreased afterward, to 35.0 ± 5.0% at 107 d (Table 1).

It was reported that the TL level in eel liver decreased more rapidly than that in eel muscle during starvation (2). In carp the visceral and muscle TL both decreased at the same rate during starvation (6). The rapid decrease of TL in both dorsal muscle and skin of the horse mackerel under starvation differed from the results for these starved freshwater fish. The tissue phospholipids (PL) in the organs of all animals, including fish, are generally retained under starvation; therefore, the phenomenon of decreasing lipid levels suggests that the depot lipids in muscle and skin of seawater fish are mobilized for energy production before those in viscera during the initial stages of starvation.

*Variations in FA composition and DHA level of TL during starvation.* The variations in FA composition of TL during starvation are shown in Table 2. The DHA content in TL of dorsal muscle increased from 13.8 to 22.0% during the initial 44 d of starvation ($Y = 0.15X + 14.6$, $r = 0.53$, $P < 0.05$, $X$: days, $Y$: DHA content), and thereafter remained constant (20.0 ± 1.0%; average between 44 and 107 d). On the other hand, the content of saturated FA decreased significantly during the initial 44 d, from 34.2 to 30.8% ($Y = -0.10X + 34.67$, $r = -0.61$, $P < 0.005$, $X$: days, $Y$: saturated FA content). The contents of monoenoic FA also decreased, from 37.2 to 32.0%, during the same period although the decrease was not significant ($Y = -0.10X + 34.81$, $r = -0.32$, $P > 0.01$, $X$: days, $Y$: monoenoic FA content). The average contents of both saturated and monoenoic FA between 44 and 107 d reached their lowest levels (saturated FA, 32.0 ± 2.4%; monoenoic FA, 32.1 ± 2.5%). The content of PUFA rose from 19.0 to 25.2%, in

**TABLE 1**
**Body Size, Condition Factor, and Crude Lipid Contents of Each Tissue from Starved Horse Mackerel[a]**

| Days starved (sample size) | Fork length (cm) | Body weight (g) | Condition factor[b] | Crude lipid in dorsal muscle (%) | Crude lipid in skin (%) | Crude lipid in viscera (%) |
|---|---|---|---|---|---|---|
| 0 ($n$ = 4) | 21.8 ± 0.4 | 143.4 ± 6.7 | 13.9 ± 0.4 | 6.8 ± 1.3 | 32.7 ± 2.1 | 56.0 ± 5.4 |
| 2 ($n$ = 4) | 22.8 ± 0.3 | 158.7 ± 7.3 | 13.7 ± 0.2 | 6.0 ± 1.3 | 25.8 ± 3.4 | 60.9 ± 5.7 |
| 16 ($n$ = 5) | 23.0 ± 0.5 | 151.3 ± 10.1 | 12.3 ± 0.1 | 3.7 ± 0.9 | 23.3 ± 4.4 | 60.4 ± 4.6 |
| 30 ($n$ = 5) | 23.0 ± 0.4 | 157.4 ± 8.6 | 12.8 ± 0.3 | 3.6 ± 1.0 | 14.6 ± 3.3 | 54.7 ± 9.6 |
| 44 ($n$ = 4) | 21.9 ± 0.3 | 124.9 ± 5.3 | 12.0 ± 0.2 | 2.3 ± 0.5 | 12.2 ± 2.3 | 51.4 ± 3.2 |
| 59 ($n$ = 4) | 22.3 ± 0.1 | 131.0 ± 3.7 | 11.7 ± 0.3 | 3.0 ± 1.0 | 7.7 ± 1.3 | 39.8 ± 6.7 |
| 74 ($n$ = 5) | 23.1 ± 0.3 | 140.0 ± 6.5 | 11.4 ± 0.1 | 2.2 ± 0.5 | 10.4 ± 1.9 | 37.6 ± 5.7 |
| 89 ($n$ = 5) | 22.7 ± 0.4 | 134.8 ± 7.5 | 11.5 ± 0.2 | 1.7 ± 0.3 | 8.8 ± 2.3 | 30.6 ± 8.9 |
| 107 ($n$ = 5) | 22.6 ± 0.2 | 135.0 ± 4.2 | 11.7 ± 0.3 | 1.8 ± 0.4 | 6.9 ± 1.7 | 35.0 ± 5.0 |

[a]Data are mean ± SE.
[b]Condition factor = 1000 × body weight/(fork length)$^3$.

**TABLE 2**
**FA Compositions (%) in Total Lipids in Muscle, Skin, and Viscera of Starved Horse Mackerel[a]**

| | Days starved (sample size) | Total saturated | Total monoenoic | Total polyenoic | 22:6n-3 |
|---|---|---|---|---|---|
| Muscle | 0 ($n$ = 4) | 34.2 ± 1.1 | 37.2 ± 0.6 | 19.0 ± 1.2 | 13.8 ± 0.6 |
| | 2 ($n$ = 4) | 35.7 ± 1.4 | 31.8 ± 1.6 | 23.9 ± 2.0 | 15.7 ± 1.6 |
| | 16 ($n$ = 5) | 32.4 ± 0.9 | 34.8 ± 1.4 | 22.8 ± 1.5 | 16.5 ± 1.4 |
| | 30 ($n$ = 5) | 31.5 ± 0.5 | 29.1 ± 2.9 | 29.1 ± 3.0 | 20.5 ± 2.6 |
| | 44 ($n$ = 4) | 30.8 ± 0.3 | 32.0 ± 2.4 | 28.0 ± 2.1 | 22.0 ± 2.1 |
| | 59 ($n$ = 4) | 30.4 ± 0.4 | 32.5 ± 2.0 | 28.9 ± 2.4 | 20.2 ± 2.2 |
| | 74 ($n$ = 5) | 31.3 ± 0.7 | 33.4 ± 2.7 | 25.7 ± 2.7 | 18.0 ± 2.4 |
| | 89 ($n$ = 5) | 31.1 ± 0.5 | 31.7 ± 3.5 | 26.8 ± 2.9 | 20.8 ± 2.6 |
| | 107 ($n$ = 5) | 32.0 ± 2.4 | 32.1 ± 2.5 | 25.2 ± 1.7 | 19.5 ± 1.8 |
| Skin | 0 ($n$ = 4) | 31.4 ± 0.6 | 36.5 ± 0.5 | 21.6 ± 0.9 | 12.8 ± 0.3 |
| | 2 ($n$ = 4) | 31.1 ± 0.4 | 36.4 ± 1.6 | 21.2 ± 1.4 | 12.4 ± 0.4 |
| | 16 ($n$ = 5) | 31.0 ± 0.7 | 39.1 ± 0.9 | 20.1 ± 1.4 | 12.5 ± 0.8 |
| | 30 ($n$ = 5) | 29.2 ± 0.9 | 38.3 ± 0.7 | 23.8 ± 2.0 | 14.8 ± 1.3 |
| | 44 ($n$ = 4) | 30.6 ± 0.2 | 36.6 ± 1.5 | 21.9 ± 1.2 | 13.8 ± 1.0 |
| | 59 ($n$ = 4) | 28.4 ± 0.0 | 39.6 ± 0.0 | 23.4 ± 0.0 | 15.6 ± 0.8 |
| | 74 ($n$ = 5) | 30.3 ± 0.5 | 38.3 ± 0.9 | 20.1 ± 0.6 | 12.1 ± 0.5 |
| | 89 ($n$ = 5) | 31.3 ± 0.2 | 39.5 ± 1.1 | 18.7 ± 0.5 | 12.5 ± 0.4 |
| | 107 ($n$ = 5) | 31.0 ± 0.4 | 40.5 ± 0.6 | 17.9 ± 0.6 | 11.7 ± 0.3 |
| Viscera | 0 ($n$ = 4) | 34.6 ± 1.3 | 34.9 ± 1.0 | 21.3 ± 1.3 | 12.7 ± 0.9 |
| | 2 ($n$ = 4) | 31.4 ± 0.5 | 35.8 ± 0.7 | 21.7 ± 1.2 | 12.8 ± 0.2 |
| | 16 ($n$ = 5) | 31.8 ± 0.8 | 38.1 ± 1.2 | 19.3 ± 1.5 | 12.0 ± 0.5 |
| | 30 ($n$ = 5) | 28.7 ± 0.2 | 35.4 ± 0.9 | 27.0 ± 0.9 | 16.6 ± 0.2 |
| | 44 ($n$ = 4) | 31.6 ± 0.3 | 34.6 ± 1.5 | 23.4 ± 1.0 | 14.6 ± 0.3 |
| | 59 ($n$ = 4) | 30.4 ± 0.3 | 38.6 ± 0.5 | 21.0 ± 0.6 | 13.7 ± 0.9 |
| | 74 ($n$ = 5) | 31.3 ± 0.6 | 36.4 ± 1.0 | 22.2 ± 0.9 | 13.2 ± 0.8 |
| | 89 ($n$ = 5) | 32.4 ± 0.4 | 37.6 ± 1.1 | 18.9 ± 1.0 | 12.5 ± 0.7 |
| | 107 ($n$ = 5) | 31.8 ± 0.6 | 39.6 ± 1.1 | 18.3 ± 0.9 | 12.1 ± 0.6 |

[a]Data are mean ± SE.

parallel with that of DHA, during the starvation test. The contents of DHA in both skin and viscera did not change significantly during the starvation test (skin, 13.1 ± 0.4%; viscera, 13.4 ± 0.5%). From those results, it was clear that horse mackerel consistently preserved high levels of n-3 PUFA, such as DHA, in their dorsal muscle, whereas both saturated and monoenoic FA were quickly metabolized for energy production under conditions of starvation. Apparently, all the FA in both skin and viscera were metabolized at approximately the same rate. Thus, the findings revealed that all the TAG FA in depot lipids were easily metabolized for energy production regardless of the FA types, but the fish maintained DHA in muscle lipids while all the FA in its subcutaneous and visceral tissues were used indiscriminantly.

*Variations in lipid class and FA compositions of TAG, PE, and PC.* Variations in the amount of lipid classes of dorsal muscles of the starved fish are shown in Table 3. The contents of each lipid class were reduced during starvation periods. In particular, neutral lipids such as SE, TAG, ST, and FFA were markedly reduced. TAG, the principal constituent of TL, declined from 5807.4 to 1348.7 mg/100 g during the 107 d of starvation ($Y = -33.74X + 4557.2$, $r = -0.61$, $P < 0.001$, $X$: days, $Y$: TAG amount), but the levels of both PE and PC decreased slowly (PE, $Y = -0.66X + 124.6$, $r = -0.41$, $P < 0.005$; PC, $Y = -0.57X + 333.2$, $r = -0.24$, $P > 0.05$, $X$: days, $Y$: amount). The respective contents fell by over half (from 177.9 to 73.0 mg/100 g for PE), and by two-thirds of the initial value (from 420.0 to 290.0 mg/100 g for PC) at 107 d of starvation, although the decrease of PC content was not significant. The rates of decline of both PE (0.55%/d) and PC (0.29%/d) in muscle were markedly lower than that of TAG (0.72%/d). Such a rapid reduction of all the FA in TAG suggests that FA in TAG not only were used as an energy source but also were supplied to PE and PC if required by cell component turnover, and for the maintenance of membrane function. TAG occupied a large percentage in TL of both skin (98.1–90.9%) and viscera (97.1–91.1%), and the levels of PE (skin: trace–1.7%; viscera: 0.4–1.7%) and PC (skin: trace–5.0%; viscera, 0.8–1.2%) were very low throughout the starvation test.

The FA compositions of TAG, PE, and PC of the dorsal muscle are presented in Table 4. The levels of both PUFA and DHA in TAG of all organs gradually decreased during the starvation period (PUFA, from 22.6 to 14.6%; DHA, from 14.4 to 11.9%). The respective regression equations were $Y = -0.07X + 23.3$ ($r = -0.72$, $P < 0.001$, $X$: days, $Y$: ratio) and $Y = -0.03X + 15.4$ ($r = -0.55$, $P < 0.005$). The percentages of saturated FA in TAG were almost constant (30.8 ± 0.52%) during starvation, and that of muscle monoenoic FA increased from 36.9 to 45.8% ($Y = 0.08X + 34.6$, $r = 0.67$, $P < 0.001$, $X$: days, $Y$: monoenoic FA ratio in TAG). Moreover, the ratios of DHA in skin TAG (12.4–11.2%) and viscera (13.0–10.3% in TAG, 22.7–17.5% in PE, and 31.0–25.5% in PC) slightly

**TABLE 3**
**Classes (mg/100 g) of the Lipids of Starved Horse Mackerel[a]**

| Days starved (sample size) | Steryl esters | TAG[b] | Sterol[b] | FFA | PE | PC |
|---|---|---|---|---|---|---|
| Muscle | | | | | | |
| 0 ($n$ = 4) | 93.8 (1.4)[c] ± 55.2 | 5807.4 (86.5) ± 1248.8 | 101.1 (1.5) ± 0.4 | 114.0 (1.7) ± 46.5 | 177.9 (2.6) ± 41.2 | 420.0 (6.3) ± 63.3 |
| 16 ($n$ = 5) | 25.2 (0.7) ± 13.8 | 2975.8 (87.4) ± 652.4 | 29.9 (0.9) ± 14.4 | 27.1 (0.8) ± 11.8 | 113.2 (3.3) ± 29.7 | 234.2 (6.9) ± 63.1 |
| 30 ($n$ = 5) | 20.3 (0.6) ± 5.1 | 3142.4 (88.2) ± 1046.5 | 16.4 (0.5) ± 3.0 | 26.6 (0.7) ± 6.5 | 42.0 (1.2) ± 6.8 | 316.7 (8.9) ± 20.0 |
| 59 ($n$ = 4) | 5.1 (0.2) ± 4.5 | 2573.4 (87.1) ± 867.6 | 9.7 (0.3) ± 5.5 | 15.2 (0.5) ± 6.0 | 78.7 (2.7) ± 15.7 | 271.8 (9.2) ± 19.2 |
| 89 ($n$ = 5) | 4.4 (0.3) ± 2.2 | 1333.4 (77.9) ± 360.1 | 1.3 (0.1) ± 1.3 | 16.3 (1.0) ± 3.5 | 72.8 (4.3) ± 10.5 | 284.1 (16.6) ± 10.1 |
| 107 ($n$ = 5) | 11.7 (0.7) ± 6.1 | 1348.7 (76.3) ± 860.0 | 15.1 (0.9) ± 7.5 | 29.9 (1.7) ± 4.1 | 73.0 (4.1) ± 9.3 | 290.0 (16.4) ± 17.6 |
| Skin | | | | | | |
| 0 ($n$ = 4) | 89.8 (0.3) ± 48.3 | 31768.7 (98.1) ± 1741.2 | 212.2 (0.7) ± 76.8 | 308.9 (1.0) ± 35.2 | Trace | Trace |
| 107 ($n$ = 5) | 55.4 (0.8) ± 11.0 | 6263.9 (90.9) ± 1702.0 | 49.6 (0.7) ± 26.3 | 59.6 (0.9) ± 24.4 | 114.9 (1.7) ± 33.8 | 343.9 (5.0) ± 66.2 |
| Viscera | | | | | | |
| 0 ($n$ = 4) | 103.7 (0.2) ± 32.8 | 54311.1 (97.1) ± 4413.9 | 284.9 (0.5) ± 95.6 | 495.7 (0.9) ± 380.0 | 250.0 (0.4) ± 166.6 | 464.7 (0.8) ± 467.5 |
| 107 ($n$ = 5) | 174.0 (0.5) ± 36.6 | 31761.0 (91.1) ± 7120.7 | 604.4 (1.7) ± 180.6 | 1286.3 (3.7) ± 570.3 | 605.7 (1.7) ± 246.0 | 414.1 (1.2) ± 282.6 |

[a]Data are mean ± SE.
[b]Includes trace DAG.
[c]Means % of total lipids.

**TABLE 4**
**FA Compositions (%) in TAG, PE, and PC in Muscle, Skin, and Viscera of Horse Mackerel[a]**

| | Days starved (sample size) | Total saturated | Total monoenoic | Total polyenoic | C22:6n-3 |
|---|---|---|---|---|---|
| Muscle | | | | | |
| TAG | 0 ($n$ = 4) | 30.2 ± 0.3 | 36.9 ± 0.5 | 22.6 ± 0.5 | 14.4 ± 0.5 |
| | 16 ($n$ = 5) | 33.1 ± 0.5 | 37.7 ± 1.1 | 19.7 ± 0.8 | 13.5 ± 0.5 |
| | 30 ($n$ = 5) | 30.8 ± 0.7 | 34.1 ± 1.4 | 23.4 ± 1.3 | 17.2 ± 1.2 |
| | 59 ($n$ = 4) | 30.6 ± 0.4 | 38.4 ± 1.1 | 19.4 ± 1.2 | 13.4 ± 0.8 |
| | 89 ($n$ = 5) | 31.5 ± 0.3 | 40.8 ± 1.1 | 16.6 ± 0.8 | 11.6 ± 0.8 |
| | 107 ($n$ = 5) | 28.9 ± 0.3 | 45.8 ± 1.6 | 14.6 ± 1.7 | 11.9 ± 1.6 |
| PE | 0 ($n$ = 4) | 23.7 ± 0.7 | 13.6 ± 1.5 | 43.3 ± 2.0 | 38.2 ± 2.1 |
| | 16 ($n$ = 5) | 28.7 ± 2.5 | 12.0 ± 1.0 | 48.9 ± 2.8 | 44.7 ± 2.8 |
| | 30 ($n$ = 5) | 20.3 ± 0.8 | 10.6 ± 0.8 | 46.4 ± 1.5 | 42.5 ± 1.1 |
| | 59 ($n$ = 4) | 18.8 ± 1.3 | 13.8 ± 0.6 | 44.8 ± 0.9 | 41.3 ± 0.9 |
| | 89 ($n$ = 5) | 20.2 ± 0.3 | 14.0 ± 1.5 | 42.5 ± 1.3 | 39.1 ± 1.1 |
| | 107 ($n$ = 5) | 20.0 ± 1.4 | 16.2 ± 1.5 | 46.7 ± 2.7 | 43.7 ± 2.7 |
| PC | 0 ($n$ = 4) | 27.5 ± 2.0 | 11.0 ± 0.6 | 50.9 ± 1.6 | 40.6 ± 2.0 |
| | 16 ($n$ = 5) | 26.3 ± 0.5 | 10.9 ± 1.1 | 52.3 ± 2.2 | 45.6 ± 1.8 |
| | 30 ($n$ = 5) | 28.6 ± 0.3 | 10.9 ± 0.9 | 51.2 ± 0.7 | 41.3 ± 0.3 |
| | 59 ($n$ = 4) | 27.7 ± 0.3 | 13.9 ± 1.7 | 49.3 ± 1.6 | 41.1 ± 1.4 |
| | 89 ($n$ = 5) | 27.3 ± 0.5 | 12.8 ± 0.5 | 51.1 ± 0.4 | 43.6 ± 0.7 |
| | 107 ($n$ = 5) | 28.5 ± 0.6 | 15.6 ± 0.7 | 47.4 ± 3.1 | 37.6 ± 3.5 |
| Skin | | | | | |
| TAG | 0 ($n$ = 4) | 31.0 ± 0.6 | 37.1 ± 0.9 | 21.0 ± 0.8 | 12.4 ± 0.3 |
| | 107 ($n$ = 5) | 27.8 ± 0.3 | 48.4 ± 2.0 | 12.1 ± 0.5 | 11.2 ± 2.0 |
| PE | 0 ($n$ = 4) | —[b] | —[b] | —[b] | —[b] |
| | 107 ($n$ = 5) | 22.6 ± 0.5 | 27.1 ± 1.9 | 30.7 ± 1.8 | 26.6 ± 1.8 |
| PC | 0 ($n$ = 4) | —[b] | —[b] | —[b] | —[b] |
| | 107 ($n$ = 5) | 22.5 ± 3.0 | 20.5 ± 1.6 | 45.6 ± 3.2 | 36.4 ± 3.9 |
| Viscera | | | | | |
| TAG | 0 ($n$ = 4) | 31.4 ± 0.7 | 35.5 ± 1.3 | 22.1 ± 1.5 | 13.0 ± 0.4 |
| | 107 ($n$ = 5) | 31.5 ± 0.8 | 46.9 ± 2.5 | 14.4 ± 1.8 | 10.3 ± 0.8 |
| PE | 0 ($n$ = 4) | 32.1 ± 1.9 | 16.4 ± 3.0 | 27.9 ± 2.5 | 22.7 ± 2.2 |
| | 107 ($n$ = 5) | 32.7 ± 3.7 | 34.0 ± 4.4 | 20.2 ± 6.2 | 17.5 ± 6.1 |
| PC | 0 ($n$ = 4) | 27.7 ± 2.6 | 17.1 ± 1.3 | 38.0 ± 5.8 | 31.0 ± 4.6 |
| | 107 ($n$ = 5) | 26.9 ± 3.9 | 30.1 ± 3.8 | 29.9 ± 8.1 | 25.5 ± 8.1 |

[a]Data are mean ± SE.
[b]Means not examined because the amounts of both PE and PC in skin tissue were negligible.

decreased during 107 d of starvation. Although the levels of PUFA and DHA in TAG of each organ were decreased, those in PE and PC of dorsal muscle were maintained at a high level during the test period (PUFA: 43.3–46.7% in PE; 50.9–47.4% in PC. DHA: 38.2–43.7% in PE; 40.6–37.6% in PC).

In contrast, the levels of both PUFA and DHA in TAG generally increased for freshwater fish, such as rainbow trout (5) and *Tilapia nilotica* (3), during starvation. From the observation that PUFA and DHA increased in carp, Takeuchi and Watanabe (6) suggested that both saturated and monoenoic FA were used preferentially as an energy source by β-oxidation and that the remaining PUFA and DHA were less likely to be catabolized compared to saturated and monoenoic FA, which are preserved in the TAG of freshwater fish (11,17,18). In the present study, the levels of both PUFA and DHA in TAG decreased, unlike results obtained with freshwater fish. This contradiction might be caused by differences in the levels of PUFA and DHA in PL between marine and freshwater fish; the horse mackerel contained high levels of PUFA (in PE, 43.3%; in PC, 50.9% at the initial stage) and DHA (in PE, 38.2%; in PC, 40.6% at the initial stage). In contrast, the freshwater fish had low levels of PUFA. For example, the ratios of PUFA and DHA in the PL were, respectively, 31.6 and 17.2% for *T. nilotica* (6), and 28.1 and 17.0% for carp (11). The PUFA in TAG might be supplied to PL by turnover, for maintaining high levels in membrane lipids, and were not used only as an energy source by β-oxidation. In the horse mackerel, the percentages of PUFA and DHA in PL were higher than those of freshwater fish because of its marine habitat and prey items. In the wild it may be able to obtain small amounts of PUFA and DHA from its prey, similar to other seawater fish, even during starvation. This might be the reason for the reduction of its PUFA and DHA in TAG.

In light of this discussion, one may conclude that DHA in the tissues of horse mackerel was preserved and accumulated during starvation and that the occurrence of consistently high levels of DHA in tissues from starved fish depended on the DHA contained by PL because DHA in TAG was decreased during starvation. Although DHA is essential and important for marine fish as well as for freshwater fish, the horse mackerel has sufficient DHA to be able to afford to use some as an energy source.

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