脂質蓄積組織の異なる魚類5種における血清リポタンパク質の特徴

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Characteristics of Serum Lipoprotein Features Associated with Lipid Levels of Muscle and Liver from Five Species of Fish

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We examined the lipoprotein features in the serum from puffer, flounder, red sea bream, amberjack, and striped jack in connection with the lipid levels of muscle and liver. Serum lipoproteins consisted of very low density lipoprotein (VLDL) and high density lipoprotein (HDL). HDL was a major lipoprotein in the five species. Species specific differences were found in the levels of VLDL. Considerable amounts of VLDL as well as HDL were present in the serum of flounder and striped jack, while the level of VLDL was extremely low in the serum of puffer. Flounder possessed unique VLDL whose main lipid was cholesteryl ester (CE) but not triglyceride (TG). The VLDLs other than that of puffer possessed apolipoprotein (apo) B- and A-I-like proteins as common components. In addition, the apolipoprotein with molecular weight (Mr) 75K was present in the VLDLs from flounder, red sea bream, and amberjack. The apolipoprotein with Mr 75K might be associated with the CE level of VLDL, and this apolipoprotein was absent in the VLDL of striped jack. The HDLs other than that of red sea bream possessed both apo A-I- and A-II-like proteins, while red sea bream possessed only apo A-I-like protein in its HDL. A close relationship was found between lipoprotein and muscle and liver lipid levels. Striped jack, with the highest level of muscle lipid, possessed the highest TG level of VLDL of the five species. Puffer, with the highest level of liver lipid, possessed the lowest level of lipoproteins. Lipoproteins synthesized in the liver seemed to be associated with the storage sites of lipid in fish.

In the circulatory fluid of animals including fish, most lipids are complexed with protein in the form of lipoproteins.[1] Lipoproteins of all animals function as major carriers of lipid and other hydrophobic compounds. Very low density lipoprotein (VLDL) is synthesized from triglyceride (TG) and apolipoproteins in the liver and secreted as TG-rich lipoprotein. TG is hydrolyzed by lipoprotein lipase during the circulation of VLDL. The hydrolytic products are absorbed in the storage sites of lipid such as mesenteric fat, subcutaneous fat, and muscle, and resynthesized into TG, the final storage product.[2] TG is considered to accumulate as droplets in the liver when the secretion of VLDL is depressed.[3] Thus the lipid accumulation among the tissues of fish is closely related to serum lipoproteins.

We reported previously that five species of fish could be clearly divided into two groups based on the lipid ratio of muscle to liver.[4] Puffer and flounder came into the group with a low lipid ratio, indicating the lipid accumulation in their liver but not in their muscle. The lipid ratios of red sea bream, amberjack, and striped jack were much higher, accumulating lipid in their muscle as well as in their liver. The differences of lipid accumulation between fish seemed to reflect their lipoprotein features. In the present study, we examined the lipoprotein features in the serum from five species of commercially important fish. The results indicate a close relationship between serum lipoprotein features and lipid levels of muscle and liver.

Materials and Methods

The same fish as shown in the previous paper,[4] *i.e.* wild puffer *Takifugu rubripes*, cultured flounder *Paralichthys olivaceus*, red sea bream *Pagrus major*, amberjack *Seriola dumerili*, and striped jack *Caranx delicatissimus*, were used as materials.

Blood was collected from the caudal vasculature of live fish and left at room temperature for several hours. The clotted blood was centrifuged at

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3,000 rpm for 15 min to obtain the serum. The serum was analyzed by density gradient ultracentrifugation.[5] An equal amount of 0.75% NaCl was gently layered over 4.5 m*l* of serum containing 2.0 g of KBr, and centrifuged at 35,000 rpm for 17 h at 15°C in a 410 rotor using an International B/60 model ultracentrifuge (DAMON/IBC). After centrifugation, 30 fractions were collected by pipette and the density was determined by refractometry.

The lipid compositions of serum and lipoprotein fractions were estimated using commercially available enzymatic kits from Kyowa Medex for TG, phospholipid (PL), free (FC), and total cholesterol (TC). The amount of cholesteryl ester (CE) was calculated using the following formula: $CE = 1.61 \times (TC - FC)$, where 1.61 is the conversion factor of cholesterol to CE. Protein concentration was determined using Bio-Rad Protein Assay Kit using bovine serum albumin as standard. For each fraction, the lipoprotein concentration was calculated from the combined contents of TG, PL, FC, CE, and protein. Density (d) regions used were VLDL, $d < 1.10$ g/m*l*; high density lipoprotein (HDL), $1.10 < d < 1.18$ g/m*l*.

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of apolipoproteins was performed according to Laemmli.[6] Electrophoresis was conducted on gradient gels (4.5–18% polyacrylamide) at 20 mA for 3 h. Lipoprotein samples contained 1% 2-mercaptoethanol and were heated to 95°C for 5 min. Protein bands on gels were stained with 0.2% Coomassie Brilliant Blue R-250. The relative molecular weights of apolipoproteins were determined by comparison with simultaneously run proteins of known molecular weight (HMW kit E and LMW kit E from Pharmacia LKB Biotechnology).

Results

Serum Levels of Protein and Lipid

The protein levels of serum from four species of fish other than amberjack were in the range of 33 to 40 mg/m*l*, while amberjack contained much more protein in the serum (Table 1). A similar relationship was found in the PL levels of serum, and the PL content of amberjack was twice as much as those of other species. FC levels ranged from 0.43 to 1.1 mg/m*l* in the five species. A reverse relationship was observed between the TG and CE contents of the serum. The ratio of CE to TG decreased in order of flounder, puffer, red sea bream, amberjack, and striped jack. The ratios for flounder and striped jack were 8.2 and 0.28, respectively. Flounder contained more CE and less TG in the serum, while the serum level of TG in striped jack was four times higher than that of CE.

Serum Lipoprotein Profiles

Fig. 1 shows the serum lipoprotein profiles from five species of fish. Serum lipoproteins consisted of VLDL and HDL in the five species. HDL was a major lipoprotein in the five species. Considerable amounts of VLDL as well as HDL were present in the serum of flounder and striped jack. VLDL was hardly found in the serum of puffer.

Table 2 summarizes the VLDL and HDL levels in the serum from five species of fish along with relative values of protein and lipid. The means of VLDL and HDL levels in the five species were 4.0 and 16.5 mg/m*l*, respectively. The standard deviations of VLDL and HDL levels of the five species were 2.9 and 7.6 mg/m*l*, respectively, suggesting that a high coefficient of variation was observed in VLDL but not HDL.

The VLDLs consisted of 10–29% of apolipoprotein and 71–90% of lipid, with the exception of puffer. Data on the VLDL of puffer seemed to be inaccurate because of its extremely low level. The main lipid component of VLDL was TG or CE in the four species. The VLDL with high contents of CE tended to decrease its TG level. The VLDL of flounder was distinguished from other VLDLs by high CE and low TG levels.

Table 1. Protein and lipid contents of serum from five species of fish

Component (mg/m*l* serum)	Wild puffer	Cultured flounder	Cultured red sea bream	Cultured amberjack	Cultured striped jack
Protein	33.41	36.13	39.74	57.04	39.03
Phospholipid	4.840	4.375	4.570	10.55	5.780
Free cholesterol	0.684	0.844	0.432	1.080	0.721
Triglyceride	0.456	0.582	0.785	3.238	3.388
Cholesteryl ester	2.043	4.765	1.975	2.673	0.932

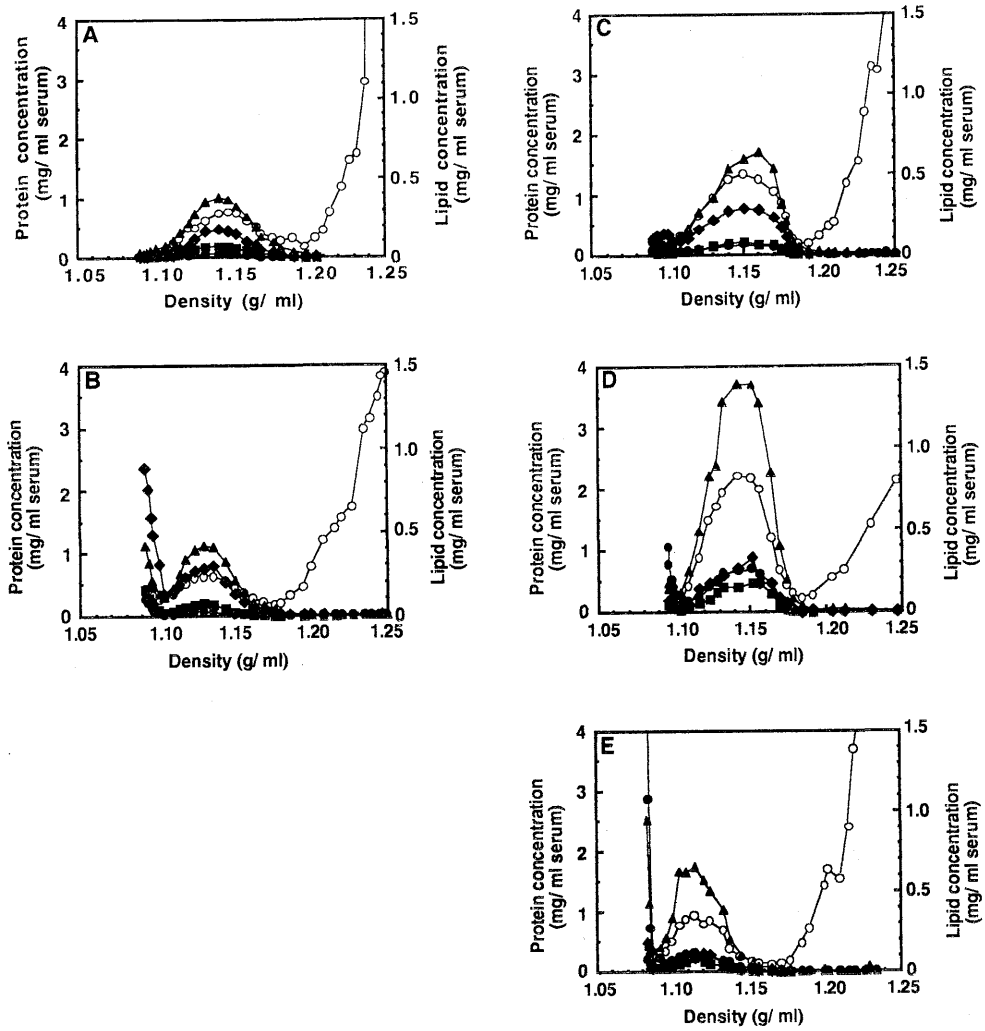

Fig. 1. Differential density gradient ultracentrifugation of lipoproteins from five species of fish.

A, Puffer; B, flounder; C, red sea bream; D, amberjack; E, striped jack; ○, protein; ●, triglyceride; ▲, phospholipid; ■, free cholesterol; and ◆, cholesteryl ester.

Table 2. Content and composition of serum lipoproteins from five species of fish

Component	Wild puffer	Cultured flounder	Cultured red sea bream	Cultured amberjack	Cultured striped jack	Mean±SD
VLDL (mg/m*l* serum)	0.63	6.87	2.20	3.17	7.02	3.98± 2.85
Apolipoprotein (%)	41.11	26.17	29.28	21.34	10.47	25.67±11.20
Phospholipid (%)	35.76	18.60	18.40	23.46	23.76	24.00± 7.06
Free cholesterol (%)	7.13	6.56	4.73	6.22	4.66	5.86± 1.11
Triglyceride (%)	4.32	4.27	15.04	37.90	56.67	23.64±23.00
Cholesteryl ester (%)	11.67	44.39	32.54	11.08	4.44	20.82±16.88
HDL (mg/m*l* serum)	11.81	11.31	16.25	29.59	13.69	16.53± 7.55
Apolipoprotein (%)	56.89	49.37	57.07	52.18	51.41	53.38± 3.44
Phospholipid (%)	25.74	26.25	24.35	31.59	32.89	28.16± 3.81
Free cholesterol (%)	4.76	4.16	2.99	3.78	3.46	3.83± 0.67
Triglyceride (%)	1.59	1.60	2.98	6.03	6.82	3.80± 2.47
Cholesteryl ester (%)	11.02	18.61	12.61	6.42	5.42	10.82± 5.30

VLDL, very low density lipoprotein; HDL, high density lipoprotein.

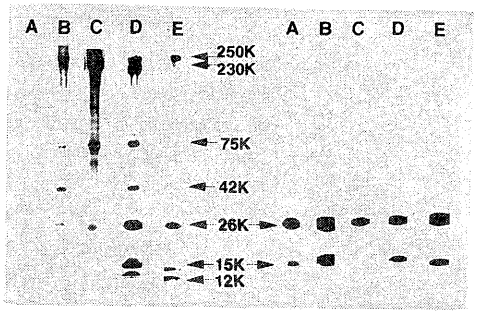

Fig. 2. Sodium dodecylsulphate-polyacrylamide gel electrophoregrams of very low density lipoprotein (left) and high density lipoprotein (right) from five species of fish.
A, Puffer; B, flounder; C, red sea bream; D, amberjack; and E, striped jack.

The HDLs consisted of 49–57% of apolipoprotein and 43–51% of lipid, regardless of species. The levels of PL and FC in the HDL were almost equal in the five species, while a reverse relationship was observed between TG and CE contents of HDL. The levels of TG in the HDL from amberjack and striped jack were higher than those from the other species.

Apolipoprotein Features

Apolipoprotein components of VLDL and HDL isolated from the five species of fish were analyzed by gradient SDS-PAGE (Fig. 2). Apolipoprotein features of VLDL differed in the five species. Apolipoproteins of VLDL from puffer were not clear because of its extremely low level. Apolipoproteins were clearly observed in VLDLs other than that of puffer. The VLDLs from four species possessed two apolipoprotein (apo) B-like proteins of molecular weights (Mr) 250K and 230K, and apo A-I-like protein of Mr 26K as common components. In addition, two apolipoproteins with Mr 75K and 42K were present in the VLDLs from flounder and amberjack. The VLDL from red sea bream possessed apolipoprotein with Mr 75K but not 42K. Striped jack possessed neither apolipoproteins with Mr 75K nor 42K in the VLDL. The VLDLs from flounder and red sea bream lacked apo A-II-like protein of Mr 15K, although VLDLs from amberjack and striped jack contained apo A-II-like protein. The HDLs other than that of red sea bream possessed apo A-I- and A-II-like proteins of Mr 26K and Mr 15K as common components, respectively. Red sea bream possessed only apo A-I-like protein in its HDL.

Relationship between Serum Lipoproteins and Lipid Levels of Muscle and Liver

In the previous paper,[4] we determined the lipid levels of muscle and liver from the five species of fish. Fig. 3 shows the relationship between lipoprotein and muscle and liver lipid levels. The levels of muscle lipid tended to increase with total lipoprotein levels. A significant relationship was found between muscle lipid and the TG contents of VLDL, suggesting that the VLDL was a carrier of TG to the muscle. Striped jack, with the highest level of muscle lipid, possessed the highest TG level of VLDL of the five species. A reverse relationship was found between liver lipid and total lipoprotein levels. Puffer, with the highest level of liver lipid, possessed the lowest level of total lipoprotein of the five species.

Discussion

More attention has been focused on the clinical importance of serum lipoprotein levels because a strong positive correlation between serum choles-

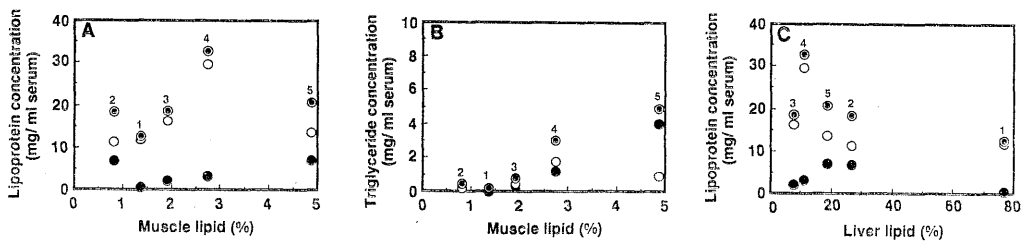

Fig. 3. Relationships between muscle lipid and lipoprotein levels (A), muscle lipid and triglyceride levels in lipoproteins (B), and liver lipid and lipoprotein levels (C).
1, Puffer; 2, flounder; 3, red sea bream; 4, amberjack; 5, striped jack; ●, very low density lipoprotein (VLDL); ○, high density lipoprotein (HDL); and ⊙, total lipoproteins (VLDL+HDL).

terol levels and the incidence of coronary artery disease has been found.[7] Mammalian serum lipoproteins are divided into four major classes based on the density of human lipoproteins[1]: chylomicrons, $d<0.94$ g/m*l*; VLDL, $0.94<d<1.006$ g/m*l*; low density lipoprotein (LDL), $1.006<d<1.063$ g/m*l*; HDL, $1.063<d<1.21$ g/m*l*. Fish seem to possess similar lipoprotein classes in their serum to mammals,[8] although the density of each fish lipoprotein class is different from that of mammals.[9] Most studies on fish lipoproteins have been carried out using rainbow trout.[10-18] Babin[14] has pointed out that the cutoff density of 1.063 g/m*l* is insufficient for the separation of LDL and HDL from rainbow trout. Density regions of lipoproteins from rainbow trout were revealed to be VLDL, $d<1.015$ g/m*l*; LDL, $1.015<d<1.085$ g/m*l*; and HDL, $1.085<d<1.210$ g/m*l*. Density regions of lipoproteins separated from the five species of fish were similar to those of rainbow trout. The VLDL and HDL from the five species were distributed to densities of less than 1.10 g/m*l* and 1.10–1.18 g/m*l*, respectively (Fig. 1). The densities of VLDL from the five species seemed to be higher than those from rainbow trout. The VLDL of rainbow trout has been characterized by the presence of apo B-, A-I-, and A-II-like proteins.[10,14,15,18] The low level of LDL has been known in seawater fish.[8,9] The VLDLs from five species possessed apo A-I-like protein as well as apo B-like-protein (Fig. 2). These results suggested that the VLDLs from five species were hardly contaminated with LDL, although the densities of VLDLs were somewhat high compared to rainbow trout.

The paper on the apolipoprotein features of fish lipoproteins is not significantly compared to other animals.[10,14,15,17-19] Of fish lipoproteins the apolipoprotein compositions of rainbow trout have been considerably investigated. Differing from mammalian VLDL, the VLDL of rainbow trout possesses apo A-I- and A-II-like proteins as well as apo B-like proteins. In addition, the apolipoprotein with Mr 76K is present in the VLDL of rainbow trout.[14,18] The apolipoprotein compositions of VLDL from amberjack were similar to those from rainbow trout, although the apolipoprotein with Mr 42K was present in the VLDL from amberjack as well as the apolipoprotein with Mr 75K and apoB-, A-I-, and A-II-like proteins (Fig. 2). The VLDL of striped jack contained apo B-, A-I-, and A-II-like proteins, but neither the apolipoproteins with Mr 75K nor Mr 42K were present in its VLDL. The apolipoprotein compositions of VLDL from red sea bream were similar to those from flounder, except for the lack of apolipoprotein with Mr 42K. The main component of VLDLs other than that of puffer was TG or CE, and the ratio of CE to TG in the VLDLs varied in the four species (Table 2). The VLDL of striped jack with the lowest ratio of CE to TG lacked the apolipoprotein with Mr 75K. The apolipoprotein with Mr 75K found in VLDLs from flounder, red sea bream, and amberjack might be associated with keeping CE at the core of VLDL. A similar behavior of apolipoprotein with Mr 76K has been recently observed in the VLDL of estrogenized trout.[18] The injection of 17β-estradiol to rainbow trout made the TG but not CE level of VLDL increase by three to eight times. A depletion of apolipoprotein with Mr 76K was found in the VLDL of estrogenized trout.

The apolipoprotein compositions of fish HDL are very similar to those of vertebrates including mammals.[8] The HDLs other than red sea bream consisted of apo A-I- and A-II-like proteins as common components, while red sea bream possessed no apo A-II-like protein in both HDL and VLDL (Fig. 2). Since human apo A-II has been known to function as an inhibitor of lecithin-cholesterol acyltransferase and hepatic lipase,[1] red sea bream without apo A-II-like protein might possess unique systems of lipid transport. Apo A-I-like protein seemed to be conserved very well throughout the HDLs of vertebrates including fish.

Vertebrates can be classified into two groups, HDL and LDL types, based on serum lipoprotein profiles.[20] Most animals belong to HDL type, while the main lipoprotein of humans is LDL but not HDL. Fish also came into the HDL type, and the HDL levels of fish were several times higher than those of other animals of the HDL type (Table 2). The high levels of HDL in fish were also predicted by the lipid compositions of serum, in which PL was a main component (Table 1). In contrast to mammals which mobilize carbohydrates, fish preferentially utilize lipid as their main source of energy.[21] This might result in the high levels of HDL in fish, whose concentrations reached 11 to 30 mg/m*l* serum (Table 2).

Lipoproteins consisted of both surface components (apolipoproteins, PL and FC) and core lipids (TG and CE). Core lipids of VLDL differed in the five species of fish. The VLDLs from amberjack and striped jack possessed TG as a

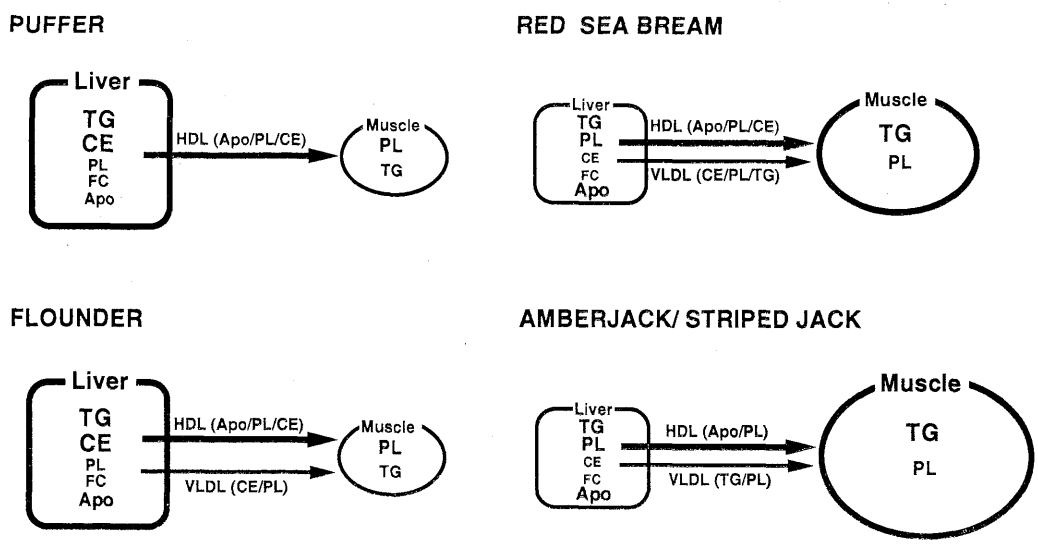

Fig. 4. A scheme proposed for lipid accumulation in five species of fish.
Bold letters show main components. Apo, Apolipoprotein; PL, phospholipid; FC, free cholesterol; TG, triglyceride; and CE, cholesteryl ester.

main lipid as well as those from other animals. CE but not TG was present as a main lipid in the VLDLs of flounder and red sea bream. There is no report on a VLDL with a high level of CE, with the exception of shark VLDL.[22] The CE level of flounder exceeded 60% of lipid. In the VLDL of flounder CE was substituted for TG and CE-rich VLDL was formed. Flounder possessed unique VLDL in the serum. Core lipids of HDL also differed in the five species. Species specific differences were found in core lipids of fish lipoproteins (Table 2).

In the previous paper,[4] we described that the lipid ratio of muscle to liver differed in the five species of fish. Lipoproteins are synthesized in the liver and secreted to the muscle, a storage site of lipid. A close relationship was found between lipoprotein and muscle and liver lipid levels (Fig. 3). VLDL levels seemed to be a determining factor by which lipid was stored in the muscle or liver, because the variation of HDL levels was smaller than that of VLDL levels in the five species. Apo B is essential for the secretion of VLDL and has very high affinity for lipid. A deficiency of apo B might result in an impaired secretion of lipid from the liver. The VLDL level of puffer was extremely low, and this might be responsible for lipid accumulation in the liver. The VLDL of flounder was a carrier of CE but not TG, suggesting that TG was accumulated in the liver without being transported to the muscle. Amberjack and striped jack with TG-rich VLDL could transport TG from liver to muscle (Fig. 4). In conclusion, lipoproteins synthesized in the liver seem to be associated with the storage sites of lipid in fish.

Acknowledgments

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References

1) A. M. Gotto, Jr., H. J. Pownall, and R. J. Havel: Introduction to the plasma lipoproteins, in "Plasma lipoproteins" (ed. by J. P. Segrest and J. J. Albers), Academic Press Inc., Orlando, FL, 1986, pp. 3–41.
2) M. A. Sheridan: Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comp. Biochem. Physiol.*, **90B**, 679–690 (1988).
3) M. Yasuhara, T. Ohama, N. Matsuki, H. Saito, J. Shiga, K. Inoue, K. Kurokawa, and T. Teramoto: Induction of fatty liver by fasting in suncus. *J. Lipid Res.*, **32**, 887–891 (1991).
4) S. Ando, Y. Mori, K. Nakamura, and A. Sugawara: Characteristics of lipid accumulation types in five species of fish. *Nippon Suisan Gakkaishi*. **59**, 1559–1564 (1993).
5) B. H. Chung, T. Wilkinson, J. C. Geer, and J. P. Segrest: Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.*, **21**, 284–291 (1980).
6) U. K. Laemmli: Cleavage of structure proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
7) M. S. Brown and J. L. Goldstein: A receptor-mediated pathway for cholesterol homeostasis. *Science*, **232**, 34–47

(1986).
8) M. J. Chapman: Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.*, **21**, 789–853 (1980).
9) P. J. Babin and J.-M. Vernier: Plasma lipoproteins in fish. *J. Lipid Res.*, **30**, 467–489 (1989).
10) E. R. Skinner and A. Rogie: The isolation and partial characterization of the serum lipoproteins and apolipoproteins of the rainbow trout. *Biochem. J.*, **173**, 507–520 (1978).
11) L. Fremont and D. Marion: A comparison of the lipoprotein profiles in male trout (*Salmo gairdneri*) before maturity and during spermiation. *Comp. Biochem. Physiol.*, **73B**, 849–855 (1982).
12) A. Rogie and E. R. Skinner: The roles of the intestine and liver in the biosynthesis of plasma lipoproteins in the rainbow trout, *Salmo gairdnerii* Richardson. *Comp. Biochem. Physiol.*, **81B**, 285–289 (1985).
13) D. Black and E. R. Skinner: Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. *J. Comp. Physiol. B*, **156**, 497–502 (1986).
14) P. J. Babin: Plasma lipoprotein and apolipoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *Biochem. J.*, **246**, 425–429 (1987).
15) P. J. Babin: Apolipoproteins and the association of egg yolk proteins with plasma high density lipoproteins after ovulation and follicular atresia in the rainbow trout (*Salmo gairdneri*). *J. Biol. Chem.*, **262**, 4290–4296 (1987).
16) D. Black and E. R. Skinner: Changes in plasma lipoproteins and tissue lipoprotein lipase and salt-resistant lipase activities during spawning in the rainbow trout (*Salmo gairdnerii* R.). *Comp. Biochem. Physiol.*, **88B**, 261–267 (1987).
17) M. A.-Jarrier, J. Burdin, L. Fremont, and M.-T. Gozzelino: Immunological evidence for common antigenic sites in high-density lipoproteins from rainbow trout and man. *Biochem. J.*, **254**, 927–930 (1988).
18) C. Wallaert and P. J. Babin: Effects of 17β-estradiol and starvation on trout plasma lipoproteins. *Lipids*, **27**, 1032–1041 (1992).
19) S. Ando and M. Hatano: Isolation of apolipoproteins from carotenoid-carrying lipoprotein in the serum of chum salmon, *Oncorhynchus keta*. *J. Lipid Res.*, **29**, 1264–1271 (1988).
20) M. J. Chapman: Comparative analysis of mammalian plasma lipoproteins, in "Plasma Lipoproteins" (ed. by J. P. Segrest and J. J. Albers), Academic Press Inc., Orlando, FL, 1986, pp. 70–143.
21) T. Watanabe: Lipid nutrition in fish. *Comp. Biochem. Physiol.*, **73B**, 3–15 (1982).
22) G. L. Mills, C. E. Taylaur, M. J. Chapman, and G. R. Forster: Characterization of serum lipoproteins of the shark *Centrophorus squamosus*. *Biochem. J.*, **163**, 455–465 (1977).