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Transport of alpha-tocopherol in Atlantic salmon (*Salmo salar*) during vitellogenesis

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Abstract

The transport of α-tocopherol was studied during vitellogenesis in Atlantic salmon that were fed diets with two levels of α-tocopherol. α-Tocopherol levels were measured in the flesh, liver, ovary and serum, and in the serum the α-tocopherol levels in the very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and very high density lipoprotein (VHDL or vitellogenin) were also measured.

Atlantic salmon store α-tocopherol mainly in their flesh because the muscle mass comprises 50% or more of live weight. During vitellogenesis the α-tocopherol content declined to about 10% of the level prior to maturation. The relative range of level of α-tocopherol in the lipoproteins was: HDL > LDL > VLDL > VHDL, irrespective of dietary levels of α-tocopherol.

From the recent knowledge on lipid transport during vitellogenesis and the present data, we hypothesize that α-tocopherol is transported from peripheral tissues to liver by HDL and further transported from liver to ovary by LDL. Vitellogenin appears to play a minor role in the transportation of vitamin E to the ovary.

Introduction

The transport mechanism of lipids between organs in fish are thought to consist of two loops, as in mammals (Sheridan 1988). One is dietary (exogenous), which includes chylomicrons and fatty acids bound to carrier protein, transporting lipids mainly to the liver, and one is an endogenous loop which transports lipids from the liver to tissues and storage sites in the form of very low density lipoproteins (VLDL). Low density lipoproteins (LDL) and high density lipoproteins (HDL) are also parts of the endogenous pathway. In addition, fish are generally oviparous and their eggs are rich in lipid yolk reserves. A female-specific, very high density lipoprotein (VHDL), vitellogenin, synthesized by the liver cells following hormonal stimulation (estrogens) plays a fundamental role in the process of gonadal growth and yolk accumulation in the oocytes. The composition of the lipids in each of the lipoproteins depend on several factors, *e.g.*, the nutritional status and stage of sexual maturity of the animal. Studies on plasma lipoproteins in fish were recently reviewed by Babin and Vernier (1989); the effects of the dietary fatty acid composition on the composition of the different serum lipoproteins have been studied in Atlantic salmon (Lie *et al.* 1993).

In mammals, α-tocopherol is transported in blood by plasma lipoproteins and there is no evi-

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dence for the existence of a specific carrier protein as has been found for the vitamins A and D (Kayden and Traber 1993). The results regarding absorption and transport of α-tocopherol were recently reviewed by Cohn *et al.* (1992) and Kayden and Traber (1993) and they concluded that many aspects of vitamin E absorption, such as the allocation to lipoproteins and delivery to tissues remain largely unknown. Information regarding transport of vitamins in fish is scanty, but according to Hung *et al.* (1982) α-tocopherol was preferentially combined with LDL in rainbow trout (*Oncorhynchus mykiss*). Further, the metabolism of LDL in rainbow trout seems to be similar to that of mammals (Gjøen and Berg 1992, 1993).

The purpose of this study was to focus on the effects of dietary α-tocopherol on the distribution and transport of α-tocopherol during vitellogenesis. This experiment is a part of a large broodstock study in which Atlantic salmon were fed diets with two dietary levels of α-tocopherol in each of three dietary lipid sources.

Materials and methods

Fish and diets

Atlantic salmon (*Salmo salar*), initial weight of 30 g (March 1988), were distributed among 12 tanks (750 in each) and fed 6 experimental diets for 24 months. During the last 12 months they were kept in net pens in the sea (2.75 × 5.5 m × 6.0 m). The diets used contained 2 levels of α-tocopherol (Low: 60 and High: 270 mg/kg) in each of three lipid sources. The basal test diet consisted of extruded dry pellets composed of fish meal (Norseamink LT, Nordsildmel, Norway), soyabean protein concentrate (Danpro A, Aarhus Oliefabrik A/S, Denmark), extruded wheat and a vitamin and mineral mix. The different lipid sources, 16% of either soyabean oil (A/S Denofa & Lilleborg, Norway), winter capelin oil (Nordsildmel, Norway) or sardine oil (J.C. Martens & Co, Norway), with or without addition of α-tocopherol acetate (Rovimix E-50, Roch, Switzerland) were coated onto the pellets after extrusion. The mean proximate composition of the diets were: dry matter (93.4%), protein (42.8%), lipid (16.9%) and ash (6.4%). The fatty acid composition of the diets were given by Lie *et al.* (1993).

Mean monthly water temperature measurements from July to September were stable at 15°C, and the monthly salinity (at 2 m depth) measurements were 25, 23 and 28 g l^{-1}, respectively.

Sampling procedure

Twenty fish from each group were sampled in March, July, September and December 1990. The fish were fasted for 24h prior to sampling and anaesthetized with a saturated benzocaine-ethanol solution. Blood samples were withdrawn from the caudal vein, allowed to clot and serum was collected after centrifugation. Pooled samples from the dietary groups of mature females (each of 3–8 fish) of fillet, liver, ovary and serum were made. In July, 2 pooled organ samples were made per dietary treatment (one per net-pen), while one pooled sample was made in September. For the lipoproteins, 1 pooled serum sample was made per dietary treatment at both samplings. All samples were stored at −80°C until further analysis. Individual data on body and organ weights were recorded, and hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated as organ weight expressed as per cent body weight.

Analytical procedures

Very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and very high density lipoprotein (VHDL) in pooled serum samples were obtained by sequential ultracentrifugal flotation (Havel *et al.* 1955; Aviram *et al.* 1983) at 4°C using a Pegasus 65 ultracentrifuge equipped with a 70-Ti rotor. The density intervals (using NaCl and KBr) and run time for separation of the lipoproteins were: VLDL, $d < 1.015$ g ml^{-1} for 20h; LDL, $1.015 < d < 1.085$ for 20h; HDL, $1.085 < d < 1.21$ for 44h and VHDL, $d > 1.21$ for 48h.

Serum and the lipoprotein fractions were tested by electrophoresis on cellulose acetate gel at room temperature for 25 min at 200V using a Gelman SEPRATEK system (Gelman instrument company,

Table 1. Mean (SEM) body and organ weights and organ indices of Atlantic salmon fed high and low dietary vitamin E levels

			Dietary vitamin E level			
	Sampling	n	Low	n	High	p[1]
Weight	July	35	3762 (131)	32	3834 (157)	ns
(g)	Sept.	11	4089 (237)	14	4088 (149)	ns
Liver	July	35	49.5 (3.1)	32	48.8 (2.9)	ns
(g)	Sept.	11	85.6 (5.4)	14	80.4 (5.6)	ns
Ovary	July	35	103 (13)	32	70 (13)	ns
(g)	Sept.	11	690 (48)	14	719 (40)	ns
HSI	July	35	1.3 (0.1)	32	1.3 (0.1)	ns
(%)	Sept.	11	2.1 (0.1)	14	2.0 (0.1)	ns
GSI	July	35	2.6 (0.3)	32	1.8 (0.3)	ns
(%)	Sept.	11	16.8 (0.6)	14	17.5 (0.6)	ns

[1]ns: not significant, according to a nonparametric Mann Whitney U test at a 5% level.

USA). No-cross-contamination of the lipoprotein classes was seen, and VHDL (vitellogenin) was only found in females verified by detection of protein bound phosphorus in sera and in the VHDL fractions from females (Waagbø and Sandnes 1988). Serum from male salmon was included as a control.

Samples of feed, fillet, liver and ovary were homogenized and small samples (0.1–0.5 g) were saponified (20 min at 100°C) using ethanol (4 ml, 96%), potassium hydroxide (5 ml, 20% w/v) and addition of pyrogallol (spatula tip), ascorbic acid (spatula tip) and EDTA (0.5 ml saturated solution). The samples were then extracted twice with n-hexane for α-tocopherol determination. A similar procedure was used for the lipoproteins (VLDL, LDL, HDL and VHDL) after ultracentrifugation and on the final precipitate (proteins).

The HPLC determination of α-tocopherol was performed with a Shimadzu (LC-9A) pump, a fluorescence detector (Shimadzu, RF-530, excitation: 289 and emission: 331 nm), the samples were injected by an autoinjector (Shimadzu, SIL-6B/9A), and separated on a column (4.6 × 150 mm) packed with silica gel (LiChrosorb, 3 μm) using 2% 2-propranol in n-hexane (v/v) as mobile phase. The amounts of α-tocopherol in the samples were calculated by using standards of dl-α-tocopherol (Merck). The methods were modified from Lambertsen (1983).

Statistics

All males, and females with GSI < 1.0 were excluded to isolate data from maturing (vitellogenic) females. The data are presented and statistically tested for intergroup differences in the biological data and organ vitamin E concentrations with regard to dietary vitamin E level, by use of a Mann Whitney U test. All tests were within a CSS: Statistica™ statistical program (Statsoft, Inc., USA 1991).

Results

There were no differences in mean body weights, organ weights or organ indices of the female Atlantic salmon with regard to vitamin E level in July or in September (Table 1). The body weights increased 7–9%, the GSI increased from approximately 2% in July to 17% in September and the mean HSI was higher in September than in July.

Table 2 details the concentrations of α-tocopherol in separate tissues and lipoproteins in July and September. The fish fed low level of α-tocopherol had significantly lower concentrations of α-tocopherol in the fillet, liver and ovary compared to fish fed a high level. A reduction of the α-tocopherol concentration was seen in all tissues from July to September and the largest reduction was seen in the liver concentration (approx. 65% in both groups). The lipid source had some effect on

Table 2. Mean (SEM) concentrations of α-tocopherol in pooled samples of organs (μg/g) and lipoproteins (μg/ml serum) in Atlantic salmon fed low and high levels of dietary vitamin E

	Sampling	n	Dietary vitamin E level		p[1]
			Low	High	
Organs					
Fillet	July	6	10.8 (0.6)	36.8 (2.1)	**
	Sept.	3	8.4 (0.6)	35.7 (2.5)	*
Liver	July	6	26.1 (2.5)	225.2 (31.1)	**
	Sept.	3	8.3 (0.8)	79.3 (46.5)	*
Ovary	July	6	59.0 (2.2)	116.4 (9.0)	**
	Sept.	3	39.3 (4.2)	91.1 (9.3)	*
Serum lipoproteins					
VLDL	July	3	13.0 (2.9)	21.0 (12.1)	ns
	Sept.	3	9.6 (3.6)	31.4 (8.2)	ns
LDL	July	3	21.4 (4.7)	43.2 (12.7)	ns
	Sept.	3	10.3 (3.8)	33.5 (4.8)	*
HDL	July	3	25.9 (4.4)	70.0 (12.4)	*
	Sept.	3	14.4 (4.1)	37.5 (3.4)	*
VHDL	July	3	3.0 (0.8)	3.9 (1.5)	ns
	Sept.	3	1.8 (0.4)	4.0 (0.5)	*

[1]ns: not significant, * $p < 0.05$, ** $p < 0.01$, according to a nonparametric Mann Whitney U test.

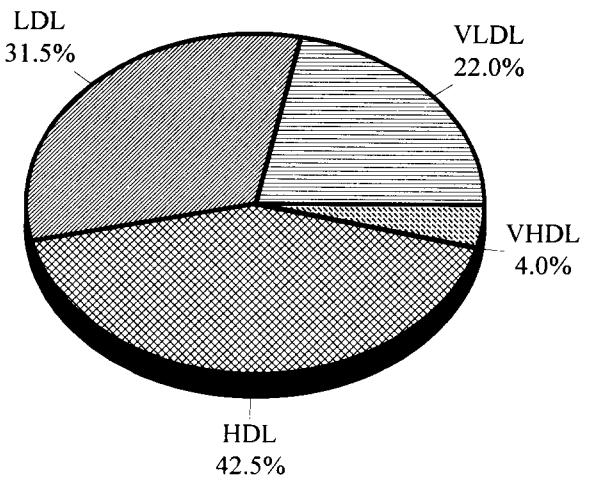

Fig. 1. Relative levels of α-tocopherol in the different lipoproteins during vitellogenesis.

the content of α-tocopherol in the livers of the fish fed the high levels of α-tocopherol.

The levels of α-tocopherol in the different serum lipoproteins varied considerably (Table 2). The lowest levels were found in vitellogenin (VHDL) in the dietary groups at both samplings and the highest levels were found in HDL. The dietary lipid sources seemed to have little effect on the α-tocopherol levels in the lipoproteins. There was a tendency (not always statistically) for the lipoproteins from salmon fed the high level of α-tocopherol to have the highest concentration of α-tocopherol compared with fish fed low levels (Table 2). The following relative range of level of α-tocopherol was found irrespective of dietary levels: HDL > LDL > VLDL > VHDL (Fig. 1). No α-tocopherol were found in the final precipitate of the centrifugation (protein fraction).

An even larger reduction in the concentration of α-tocopherol were seen in the liver and particularly in the fillet during the final part of vitellogenesis from September to November/December (Waagbø *et al.*, unpublished). Fig. 2 shows the net amount (mg) of α-tocopherol in the fillet (representing 50% of the body weight), liver and ovary during vitellogenesis for the fish led low (a) and high levels (b) of α-tocopherol. The figure illustrate the massive transfer of α-tocopherol from the fillet to the ovary during the last part of vitellogenesis after feeding was terminated in September.

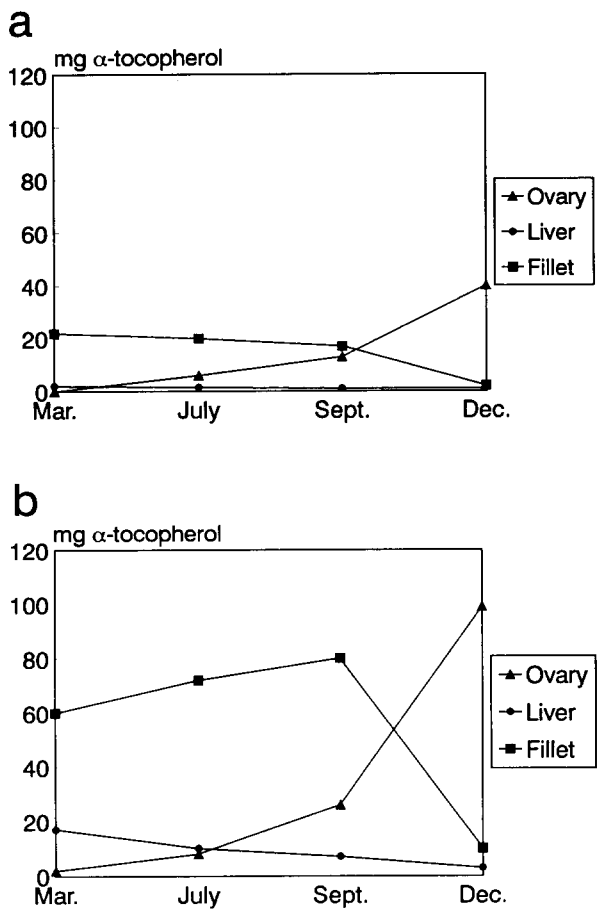

Fig. 2. The total content (mg) of α-tocopherol in tissues during vitellogenesis in Atlantic salmon fed low (a) and high level (b) of α-tocopherol.

Discussion

Vitellogenin is synthesised in the liver under the control of estradiol produced by the follicle cells in the ovaries (De Vlaming *et al.* 1980). Maximum blood levels of vitellogenin are reached prior to the final egg maturation. The lipoproteins in the present study were collected early and in the middle of the vitellogenesis, as indicated by a low and high GSI, respectively (mean GSI at stripping in December was 26.3%).

The α-tocopherol levels in the fillet, liver and ovary reflected significantly the dietary level of α-tocopherol. The highest concentration was found in liver of salmon fed the highest level of α-tocopherol, however, the largest total amount of α-tocopherol was present in the fillet (Fig. 2). According to Gallo-Torres (1980), the adipose tissue, liver and muscle are the major storage depots for tocoperhol in mammals, but the adipose tissue seems to contain the largest non-exchangeable pool of tocopherols. Recent knowledge (reviewed by Kayden and Traber 1993) suggests, however, that α-tocopherol in adipose tissue is more readily available than previously thought.

Atlantic salmon does not eat during the last part of the gonadal development. In this study, feeding was terminated in September and mature fish were stripped in December. The final and main deposition of nutrients in the egg is thereby dependent on the maternal body stores. The main part of the α-tocopherol in the ovary was derived from the fillet, whereas minor amounts seem to have originated from the liver. It is generally assumed that α-tocopherol in tissues exists as structural components of membranes. According to Gallo-Torres (1980) α-tocopherol may be stored in the oil droplet in the lipid-storing adipocytes, but these depots are not readily bioavailable.

Atlantic salmon store lipids largely in the fillet and the lipid levels were reduced to about 50% during vitellogenesis (Waagbø, unpublished), whereas the α-tocopherol levels were reduced to about 10% compared to the levels before maturation. These results suggest a bioavailable pool of α-tocopherol in the fillet and a specific transport from fillet to the developing ovary.

α-Tocopherol was present in all lipoproteins, but not detected in the final precipitate after fractional centrifugation of serum. The summation of α-tocopherol in the lipoprotein fractions constituted all α-tocopherol present in native serum. This indicate that there is no specific plasma transport system for vitamin E in fish. The absorption of α-tocopherol from the intestinal tract is suggested to follow the same intraluminal mebrane and intracellular events as has been described for dietary lipids (reviewed by Cohn *et al.* 1992). In the circulation, chylomicrons are catabolized by an endothelial-bound lipoprotein lipase which include transfer of tocopherols to tissues (Traber *et al.* 1985). Excess of surface components, including tocopherols, may be

transferred to HDL during the formation of chylomicron remnants, which are subsequent absorbed by the liver (Kayden and Traber 1993).

The highest relative levels of α-tocopherol were found in HDL and LDL, but HDL is the dominating lipoprotein in Teleostei (Babin and Vernier 1989). These aspects suggested that HDL in addition to LDL are the main transport lipoproteins for α-tocopherol in Atlantic salmon, even during vitellogenesis. Hung *et al.* (1982) reported that α-tocopherol circulates in the plasma preferentially combined with LDL in rainbow trout, but no other information regarding the transport of α-tocopherol in fish is available. Other lipid soluble substances such as astaxanthin are transported by HDL and VHDL in maturing chum salmon (*Oncorhynchus keta*) (Nakamura *et al.* 1985; Ando *et al.* 1985, 1986)

According to Cohn *et al.* (1992), LDL-α-tocopherol may be delivered to peripheral tissues by both receptor-dependent pathways as well as separate uptake without concomitant LDL uptake. Hepatocytes of rainbow trout can take up the majority of circulating LDL by both specific and nonspecific mechanisms (Gjøen and Berg 1993).

The depletion of lipids in fillet during the last part of vitellogenesis suggest a reverse transport from muscle to the liver, probably by HDL. The α-tocopherol concentration declined at most in the liver from July to September, concomitantly with an increase in liver weight and HSI. Thus, the liver α-tocopherol concentration may reflect the decrease in HDL tocopherol (45%) in both groups, regardless of dietary α-tocopherol level, as the liver represent the predominant site for HDL uptake (Cohn *et al.* 1992). This may imply a role for HDL in the concomitant reverse α-tocopherol transport from peripheral tissues to the liver. From the liver, α-tocopherol may be transported by LDL to the ovary although other tissues, such as the adrenal glands have high LDL receptor activity (Kayden and Traber 1993).

It may be therefore be suggested that α-tocopherol are transported by HDL to the liver, and further transported from liver to ovary by LDL in maturing Atlantic salmon. Vitellogenin seems to play a minor role in the transportation of vitamin E to the ovary. Further studies are needed to support the suggested transport pattern.

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