Age-related, sex-related, and seasonal changes of plasma lipoprotein concentrations in trout

Charlotte Wallaert and Patrick J. Babin[1]

Unité de Recherche Associée 1134 du Centre National de la Recherche Scientifique, Université Paris-Sud, Bât. 447, 91405 Orsay, France

Abstract Seasonal variability in physiological parameters can be attributed to seasonal variations in environmental factors and/or to the consequence of the presence of endogenous circannual rhythms. In the current study we have measured plasma levels of lipids and of the different lipoprotein classes in fasting trout (*Oncorhynchus mykiss*) between the ages of 5 and 44 months. Independent of age and sexual maturity, a circannual variation in the low density lipoprotein concentration between 250 and 1300 mg/dl was demonstrated in both sexes. These seasonal fluctuations might be controlled by an endogenous biological clock synchronized by the photoperiod. The lipoprotein profile of trout is dominated by high density lipoproteins as early as the first months of life. Their concentration increases progressively during sexual maturation from about 1200 mg/dl in juveniles to about 2500 mg/dl during spermiation or at the moment of ovulation. This increase is highly significantly correlated with the increased concentration of testosterone occurring in both sexes during sexual maturation. The concentration of very low density lipoproteins increases substantially, from about 150 mg/dl to a maximal concentration of 800 mg/dl in females and 1100 mg/dl in males, during the deposit phase of lipid reserves which precedes the rapid increase in the gonadosomatic ratio. In the course of rapid ovarian growth, vitellogenin appears in the plasma of females and reaches a concentration of 2200 mg/dl 1 month before ovulation. From these results it is concluded that season and reproductive cycle are the two main factors affecting basal plasma lipid and lipoprotein levels in trout. Environmental factors such as photoperiod or endocrine factors such as the concentration of steroid hormones can be correlated and/or involved in the regulation of these quantitative variations. These results also suggest the presence of an endogenous biological clock able to exert an independent effect on plasma lipid and lipoprotein levels.—**Wallaert, C., and P. J. Babin.** Age-related, sex-related, and seasonal changes of plasma lipoprotein concentrations in trout. *J. Lipid Res.* 1994. **35:** 1619–1633.

Supplementary key words aging • biological clock • endogenous rhythm • photoperiod • plasma lipids • seasonal variations • sexual maturation • steroid hormones

Circadian and circannual variations of physiological parameters are believed to be at least partly due to endogenous biological rhythms and have been detected in humans for several biochemical components, including plasma lipid and lipoprotein concentrations (see refs. 1 to 5 for reviews). Steady-state plasma lipoprotein levels reflect a balance between processes facilitating their accumulation and processes promoting their catabolism. As multiple factors determine the efficiency of these processes, several environmental factors in addition to dietary factors (see ref. 6 for review) may influence plasma lipid and lipoprotein levels. The seasonal variability observed in humans can probably be partly attributed to seasonal variations in these environmental factors. However, the degree and the molecular mechanisms to which endogenous biological rhythms exert an independent effect on variations in lipid and lipoprotein concentrations are presently unknown.

Numerous fish species are useful animal models for studying lipid metabolism and transport. In contrast to mammals which mobilize carbohydrates, fish preferentially utilize lipids as their main source of energy. Cholesterol and other lipids are transported in the blood of fish by different lipoprotein classes, whose basic molecular organization and role in lipid metabolism are similar to those described in mammals (see ref. 7 for review). The different classes of plasma lipoproteins and their apolipoproteins have been characterized in a detailed manner in a number of fish species, in particular in trout (7–12). The use of standards, applied to mammals and humans in particular, classifies fish as hyperlipidemic and hyperlipoproteinemic (7). Different factors such as age, sex, diet, temperature, and sexual maturation may affect the plasma cholesterol concentration in fish (7, 13). In trout, this concentration may vary from about 100 to 1000 mg/dl, depending on the studies (7). Broad seasonal variations of blood cholesterol levels related to sexual

Abbreviations: ANOVA, analysis of variance; apo, apolipoprotein; BSA, bovine serum albumin; d, density; EDTA, ethylenediamine tetraacetic acid; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; SDS, sodium dodecyl sulfate; VTG, vitellogenin.

[1]To whom correspondence should be addressed.

maturation have thus been demonstrated in a number of fish species (7). However, available data on the quantitative variations of lipids and of the different classes of plasma lipoproteins related to age, season, and to the reproductive cycle are rare, fragmentary, and occasionally contradictory. Variations in the concentration of the different lipoprotein classes linked to the nutritional status (7, 14) or to the annual reproductive cycle have been characterized in fish (15–20).

It has been demonstrated for a number of annually breeding vertebrates that under natural conditions the annual change in photoperiod continuously entrains an autonomous endogenous circannual clock, thus ensuring that maturation and spawning occur at the optimal season for the survival of the species (see ref. 21 for review). This endogenous circannual oscillator is present in trout and can be dissociated from the neuroendocrine mechanisms controlling sexual maturation (22). This demonstration prompted us to investigate in detail in a long-term study the variations of plasma lipid and lipoprotein levels in male and female trout. This work has enabled us to dissociate between the ages of 5 and 44 months factors such as age, season, sex, and reproductive cycle affecting basal lipoprotein levels and to identify some environmental and endocrine factors that could be correlated and/or involved in the regulation of these quantitative variations. In particular, we provide evidence for a circannual variation in the low density lipoprotein (LDL) concentration that could be controlled by an endogenous biological clock synchronized by the photoperiod. We have also found that the reproductive cycle in both sexes dramatically alters the plasma concentration of very low density lipoproteins (VLDL) and high density lipoprotein (HDL), and the appearance of a new lipoprotein, vitellogenin (VTG), in the plasma of females.

MATERIALS AND METHODS

Animals and diets

Male and female rainbow trout (*Oncorhynchus mykiss*) used in this study were the offspring of a single male and female and were exposed to the natural photoperiod and seasonal variations of water temperature throughout the 44 months of the experiment (**Fig. 1**). Trout is a poikilothermic vertebrate that remains active at low temperature. Animals of both sexes were kept together in our breeding tanks at the University Paris-Sud in Orsay. Trout age was defined with regard to the fecundation day. Blood sampling was made from April 1990 to July 1993, between the ages of 5 and 44 months. Since June 1991, blood was sampled every 4 weeks on 16 trout randomly selected in the population. Trout were individualized by a specific identification of skin spots on the head. Those 19-month-old trout were juvenile and so their sex was indeterminable. In trout, a great percentage of males and a low percentage of females are sexually precocious at 2 years. The whole of the population is sexually mature at 3 years. Among the 16 trout, there were 9 males including 5 precocious males and 7 females including 1 precocious. Three trout died during the experiment.

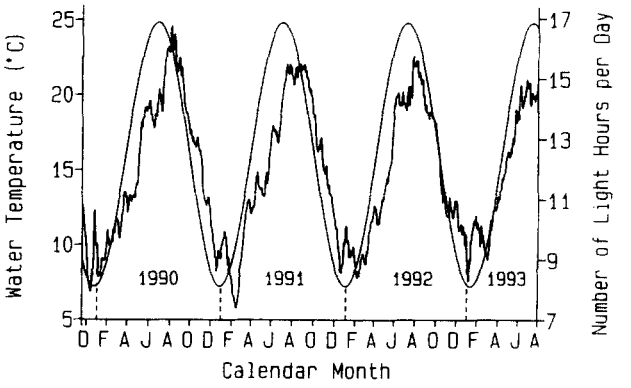

Fig. 1. Circannual changes in the photoperiod (regular line) and water temperature (irregular line) from the moment of fertilization until the end of the experiment.

The animals were given free access to a standard granule diet (Aqualim, Nersac, France) daily until they reached a body weight of 10 g. Then they were given free access to food three times a week (Monday, Wednesday, and Friday). According to the manufacturer, the diet composition was (percentage by weight), protein 45%, fat 11%, nonextractible nitrogenous 21%, ash 11%, fiber 2%, humidity 10%. The diet composition was held constant regardless of the water temperature at which the animals were raised. Trout were weighed at each sampling. Growth is continuous in fish and the body weight gain of our male and female trout was regular until 36 months. Then a dimorphism occurred between both sexes due to the low weight gain of males during spermiation (data not shown).

The absence of complete natural reproductive behavior in breeding conditions results in females retaining ova in the general body cavity after ovulation. The absence of spermatozoa release in males was also observed. The ova and spermatozoa can be expelled from the fish by gentle stripping. During the annual reproductive cycle, the presence of spermiating males or ovulating females was verified at each sampling. In such cases, ova present in the body cavity or spermatozoa that could be released from the testicles were evacuated by stripping. In females, stripping was done to avoid the intensive yolk resorption after massive intraovarian follicular atresia of non-ovulated oocytes. Follicular atresia results in the appearance of egg yolk proteins combined with HDL in the plasma (11).

Blood samples

In trout, the absorption peak of dietary lipids shown by the postprandial variations in the triacylglycerol concentration occurred later at low than at high temperature and may be offset by as much as several tens of hours, e.g., 48 h at 6°C vs. 10 h at 22°C (23). The triacylglycerol concentration and thus the plasma concentration of triacylglycerol-rich lipoproteins in all cases return to basal level 4 days after feeding, regardless of the acclimatization temperature of the animals (23). Trout were anesthetized with ethyleneglycol monophenyl ether (0.3 ml/l). Blood was drawn after a 7-day fast over ethylenediamine tetraacetic acid (EDTA) and NaN_3 (3 and 0.15 mg/ml blood, respectively, dissolved in 0.15 M NaCl, final pH = 7.4) by cardiac puncture with a fine catheter (diameter 0.58 mm) and kept at 4°C throughout the procedure. Plasma was obtained by centrifugation (3,000 *g*, 10 min). The estimated whole body blood volume of trout is 4–4.5 ml per 100 g weight (24, 25). In these experiments, the volume of blood collected at each point was less than 10% of the total body volume. The fish were sampled at the same time of the day (10–12 AM) to avoid interference by circadian rhythms in the measured parameters.

Plasma was frozen at −80°C for subsequent chemical analysis or stored up to 48 h at 4°C for lipoprotein fractionation. Frozen storage of trout plasma is unsuitable for subsequent quantitation of lipoprotein classes as separated by density gradient ultracentrifugation (26). Lipoproteins of intermediate density between HDL and LDL appeared after freeze-thawing. This is related to the very high concentration of HDL in trout plasma and to their aggregation after frozen storage.

Lipoprotein fractionation and analysis

Fractionation of whole plasma lipoprotein classes and proteins was carried out by a refinement of a density gradient ultracentrifugation procedure previously described (12). Discontinuous six-step density gradients were prepared with NaBr solutions, containing 0.05% EDTA (pH 7.4). Successive densities were (from top to bottom of tube): 1.006 g/ml (1.1 ml); 1.019 g/ml (2.5 ml); 1.063 g/ml (3.5 ml); 1.210 g/ml) (2.5 ml); 1.310 g/ml (0.85 ml of plasma at 1.310 g/ml, adjusted to this density with solid NaBr, and completed with a 1.310 g/ml NaBr solution to give a final volume of 1 ml) and 1.386 g/ml (0.8 ml). Six percent of the plasma volume is assumed to consist of macromolecules, and the remaining 94% is equivalent to a salt solution of density 1.015 g/ml. Instead of the adjusted plasma sample, control gradients were prepared using an NaBr solution of density 1.015 g/ml adjusted to 1.310 g/ml with solid NaBr. The gradients were then placed in a Beckman (Palo Alto, CA) SW 41-Ti swinging bucket rotor (average radius 110.2 mm) and centrifuged at 40,000 rpm for 28 h at 10°C in a Beckman L8-70 ultracentrifuge, without braking at the end of the run.

The centrifuge tube containing the separated lipoproteins was punctured at the bottom and connected to the fractionation system filled with a 1.386 g/ml NaBr solution. The protein and lipoprotein profile was recorded by continuously monitoring absorbance at 280 nm with a UV monitor (LKB 2238 Uvicord SII, LKB, Bromma, Sweden) as the lipoproteins were pumped from the centrifuge tube (collection speed 0.6 ml/min, chart speed 10 mm/min). One arbitrary unit of absorbance was taken as the optical density of a 1 mg/ml solution of bovine serum albumin (BSA; Cohn fraction V, Sigma Chemical Co., St. Louis, MO). At the end of the fractionation, distilled water was carefully deposited onto the upper layer of the tube until VLDL was completely eluted. Fractions were collected with a fraction collector. Quantitative lipoprotein profiles were determined based on total lipoprotein concentration and the volume of the combined tube fractions (60 fractions, mean volume 300 μl). The lipoprotein concentration was expressed as lipoprotein-cholesterol or was calculated from the expression: total lipoproteins = cholesteryl esters + free cholesterol + triacylglycerol + phospholipids + proteins. The lipoprotein concentrations obtained in each density gradient fraction were summed between the density intervals defining the different classes of lipoproteins in trout (11, 12, 14). Density regions used were d 1.210–1.310 g/ml for VTG (subfractions 10 to 17), d 1.085–1.210 g/ml for HDL (subfractions 18 to 28), d 1.015–1.085 g/ml for LDL (intermediate density lipoproteins (IDL) + LDL, subfractions 29 to 43), d < 1.015 g/ml for VLDL (subfractions 44 to 60). VTG is a very high density lipoprotein specifically present in females during vitellogenesis, i.e., the accumulation of vitelline reserves in growing oocytes (27), and contains 2% cholesterol in trout (28). VTG-cholesterol and VTG concentrations were determined by subtracting at each blood sampling the mean of total cholesterol or of lipids and proteins present in the density interval 1.210–1.310 g/ml in males from the values recorded in each female in the same density interval. Lipoprotein concentrations were expressed as milligrams lipoprotein total cholesterol or milligrams lipoprotein per deciliter of plasma after correction for the dilution introduced by the anticoagulant. The lipoprotein concentrations in each plasma were normalized after correction for the recovery of plasma lipids or proteins in the density gradient fractions relative to whole plasma. The mean percent recoveries after the entire density gradient fractionation procedure were: 92.09 ± 0.95% for total cholesterol, 88.61 ± 1.17% for free cholesterol, 90.28 ± 1.32% for cholesteryl esters, 88.58 ± 1.45% for triacylglycerol, 90.5 ± 1.33% for phospholipids, and 89.63 ± 1.14% for proteins (n = 31). The analytical precision of the density gradient quantitation method was determined from blind dupli-

cate pairs of plasma samples and was defined as the mean absolute difference between duplicates divided by the mean of the duplicates times 100 (seven duplicate pairs analyzed). The precision values for lipoprotein-cholesterol concentrations were: total cholesterol 0.83%, VLDL-cholesterol 13.41%, LDL-cholesterol 4.47%, HDL-cholesterol 2.36%.

The apolipoprotein compositions of the major classes of trout lipoproteins and the similarities of trout apolipoproteins with their human counterpart have recently been described (7, 11, 12, 29–31). Apolipoproteins were electrophoresed under reducing conditions in sodium dodecyl sulfate (SDS)/glycerol/polyacrylamide slab gels using a linear gradient of 3.5–15% polyacrylamide and 8–12% glycerol (11). The relative molecular mass (M_r) values of trout apolipoproteins were determined as previously described (11) by comparison with simultaneously run proteins of known M_r (MW-SDS-70L and MW-SDS-200 molecular weigh marker kits from Sigma Chemical Co.). Gels were subsequently fixed and stained using a highly sensitive Coomassie Blue G-250 procedure (32).

Chemical analysis of plasma lipids and lipoproteins

The lipid compositions of plasma and of the lipoprotein fractions were evaluated by microassay (33) using commercially available enzymatic kits from Boehringer Mannheim (Meylan, France) for total cholesterol and free cholesterol and from Wako-Unipath (Dardilly, France) for triacylglycerol and phospholipids. Density gradient fractions (100 μl) or plasma (5 μl) were pipetted in duplicate into wells of microplates (Nunc Polysorp) and mixed, respectively, with 100 or 200 μl of either enzymatic lipid reagent. The microplates were incubated at room temperature for 20 min and absorbances were read at 492 nm in a Tittertek Multiscan Plus microplate reader (Labsystems, Helsinski, Finland). The enzymatic method used to assay triacylglycerol was, in fact, the assay of total glycerol (triacylglycerol glycerol + free glycerol) in the sample. The free glycerol mean concentration in trout plasma accounts for about 6% of total plasma glycerol (23). Triacylglycerol glycerol concentration in plasma was corrected with this free glycerol mean concentration. The amount of cholesteryl esters was calculated using the formula: cholesteryl esters = 1.7 × (total cholesterol − free cholesterol). The plasma cholesteryl esters and triacylglycerol concentrations were calculated by using 288 as the average molecular weight of the trout plasma fatty acids (14). The protein concentrations were determined in duplicate with a modified Lowry assay (34) adapted to microplates, using BSA as a standard. The microassay has an intra-assay coefficient of variation of 1.49% for total cholesterol, 1.6% for free cholesterol, 1.76% for triacylglycerol, 1.45% for phospholipids, and 1.3% for proteins, while the inter-assay coefficient of variation was 3.81% for total cholesterol, 3.5% for free cholesterol, 4.14% for triacylglycerol, 1.92% for phospholipids, and 4.39% for proteins. The concentrations of the different plasma parameters are expressed as milligrams per deciliter of plasma after correction for the dilution introduced by the anticoagulant.

Hormone assay

The levels of plasma 17β-estradiol and testosterone were measured by radioimmunoassay (RIA Kit, BioMerieux, Marcy-l'Etoile, France) as previously described (35). The anti-17β-estradiol used had a 10% cross-reaction (B/B_o = 0.5) with 6-oxo-17β-estradiol, 1% with estrone, and 0.8% with 17-estradiol. The anti-testosterone used had a 48% cross-reaction with 5α-dihydrotestosterone, 7.5% with 5β-dihydrotestosterone, and 2.6% with 5α-androstane-3,17 diol.

Statistical analysis

Data are presented as means ± SEM and were tested for statistical significance by analysis of variance (ANOVA) followed by an evaluation employing the Tukey-Kramer's multiple range test. Data were analyzed separately for the three groups of animals studied, precocious males, males, and females. Results were evaluated with the Statistical Analysis System (SAS) computer programs (36). The P value chosen for statistical significance was 0.05. For linear regression analysis, a logarithmic transformation was performed for variables (i.e., 17β-estradiol and testosterone) that were not normally distributed.

RESULTS

Variations in plasma lipid concentrations

The concentration of plasma lipid parameters determined after a 7-day fast was modified under the effect of aging, season, and reproductive cycle. Total plasma cholesterol was significantly increased ($P < 0.0001$) from 184.1 ± 10.5 mg/dl at 5 months of age to 534.2 ± 62.1 mg/dl in males and to 474.8 ± 38.9 mg/dl in females at 43 months of age (**Fig. 2**). This was related to a concomitant increase ($P < 0.0001$) of cholesteryl esters from 192.1 ± 9.4 mg/dl to 573.5 ± 47.4 mg/dl in males and to 482.5 ± 79.1 mg/dl in females and of free cholesterol ($P < 0.0001$) from 66.5 ± 3.1 mg/dl to 197.8 ± 11.2 mg/dl in males and to 175.4 ± 19.3 mg/dl in females. The percentage of esterified cholesterol in the plasma relative to total cholesterol was maintained around 65% (Fig. 2) in both sexes, except for females during the 2 months preceding ovulation, which occurred in November at the age of 36 months. For example, this percentage was significantly different between males and females in September (63 ± 1.2% vs. 49.8 ± 2.5%, $P < 0.005$). Similarly, the triacylglycerol concentration was significantly increased ($P < 0.001$) from 170.3 ± 15.6 mg/ml at

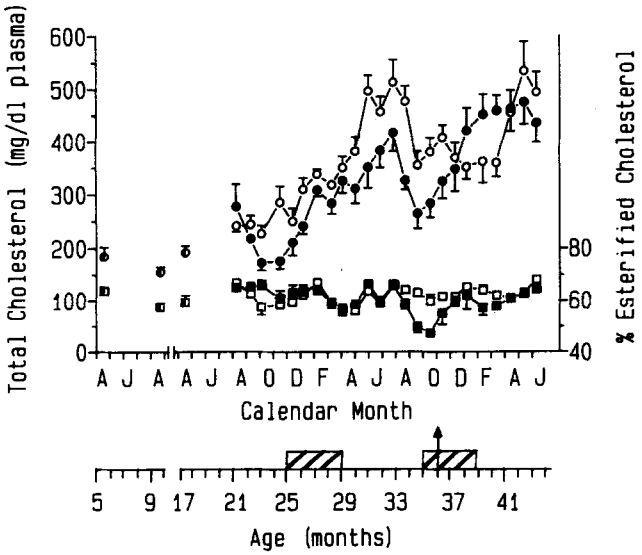

Fig. 2. Variations in plasma cholesterol concentrations (circle) and percentage of esterified cholesterol in the plasma relative to total cholesterol (square) related to age, season, and to the reproductive cycle in trout. The animals used were obtained from two identical progenitors and up to the age of 17 months they were not separated and their sex could not be determined. Starting at 19 months, trout were individualized and their sex could subsequently be determined after sexual maturation. The values are means ± SEM (SEM within data points are not shown) for trout of both sexes (●, ■) (n = 12), for male trout (○, □) (n = 8 or 9) and for females (●, ■) (n = 5 to 7). The hatched rectangle indicates the period of spermiation and the arrow indicates the moment of ovulation.

5 months to 346.3 ± 29.1 mg/dl in males and to 377.3 ± 44.6 mg/dl in females at 43 months. The phospholipid concentration significantly increased ($P < 0.001$) from 442.5 ± 22 mg/dl at 5 months to 892.6 ± 37.8 mg/dl in males and to 967.3 ± 127.4 mg/dl in females at 43 months.

During the first female reproductive cycle, between the ages of 24 and 36 months, total plasma cholesterol increased from 172.6 ± 15.1 mg/dl in November to 416.6 ± 36.1 mg/dl in July ($P < 0.001$) and then decreased to 263.9 ± 28.2 mg/dl in September (Fig. 2). At the end of the annual reproductive cycle, the total cholesterol concentration observed during ovulation in November was higher (324.8 ± 32.8 mg/dl) than the mean observed in the previous November. This may be due to a progressive increase in the total plasma cholesterol concentration with age. During sexual maturity of males, similar variations of total plasma cholesterol concentrations were observed. ANOVA applied to the data collected during the experiment revealed no significant differences in total cholesterol concentrations between precocious males and males. During the reproductive cycle of males, between the age of 29 to 39 months, total plasma cholesterol increased significantly ($P < 0.001$) from 325.7 ± 23.4 mg/dl in April to 512.9 ± 43 mg/dl in July and then decreased to 354.2 ± 26.5 mg/dl in September. The total cholesterol concentration then remained relatively stable until February, including the period of spermiation. After spermiation or ovulation, total cholesterol increased during the first part of the next reproductive cycle, as described for the first reproductive cycle.

Correlations between plasma lipid concentrations were tested by linear regression analysis. As no significant differences were observed between sexes, the calculations are based on the mean values for both sexes at each time point of the experiment. A very significant positive correlation was found among the concentrations of all plasma lipids, free cholesterol, cholesteryl esters, triacylglycerol, and phospholipids ($r > 0.60$, $P < 0.0001$, n = 50). The most highly correlated plasma lipid concentrations in fasting trout were free cholesterol versus cholesteryl esters ($r = 0.854$, $P < 0.0001$) and free cholesterol versus triacylglycerol ($r = 0.823$, $P < 0.0001$).

Variations in plasma lipoprotein concentrations

The efficiency of the separation of the different trout lipoprotein classes obtained by our standardized fractionation procedure is illustrated in **Fig. 3** by a representative pattern obtained upon electrophoresis of the protein moieties of lipoprotein subfractions. As pointed out previously (8–12), a density of 1.085 g/ml is adequate as a cutoff between the trout LDL and HDL. There are two major apolipoproteins of M_r 25,000 (apoA-I) and 13,000 (apoA-II) in HDL and the existence of a predominant type of M_r 240,000 apoB together with an apolipoprotein of M_r 76,000 and trace amount of apoA-I in LDL.

Complete lipoprotein profiles were obtained by simultaneously fractionating 10 μl of plasma of 5-month-old trout (mean body weight 2.38 ± 0.77 g (±SD)) or 18-month-old trout from another cohort (mean body weight 248 ± 42 g) by density gradient ultracentrifugation. After a 4-day fast, the relative percentage of each lipoprotein class, determined by integrating peak surface areas of absorbance profiles at 280 nm (not corrected for the different chromogenicities of the different lipoprotein fractions), was similar for 5- and 18-month-old trout, 48.64% versus 50.88% for HDL, 38.15% versus 36.45% for LDL, and 13.21% versus 12.67% for VLDL. Relative weight percentages of the lipoprotein fractions presented in **Table 1** revealed the predominance of HDL in the lipoprotein profile. For example, HDL account for 77.4% and 74.4% of total lipoproteins in 25-month-old juvenile males and females, respectively. This predominance of HDL for the four trout subpopulations, precocious and nonprecocious males and females, is well illustrated in the profiles of **Fig. 4.**

The observed changes in plasma concentrations of the different lipids previously described with age, season, and reproductive cycle revealed changes in plasma lipoprotein concentrations. Starting at the age of 1 year in sexually

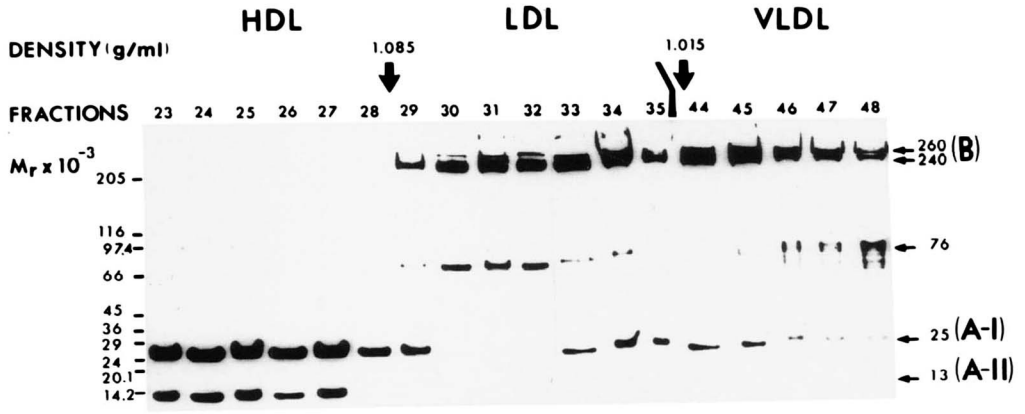

Fig. 3. Electrophoretic patterns in SDS/glycerol/polyacrylamide gradient gel slabs of the apolipoproteins of trout lipoprotein subfractions isolated by gradient density ultracentrifugation. Whole trout plasma lipoprotein classes and proteins were fractionated in 60 successive subfractions as a function of density (see Methods). Using this procedure, trout HDL (d 1.210–1.085 g/ml) were recovered between subfractions 18 to 28, LDL (LDL + IDL, d 1.085–1.015 g/ml) between subfractions 29 to 43, and VLDL (d < 1.015 g/ml) between subfractions 44 to 60. On the left hand side of the figure, the relative molecular weights ($M_r \times 10^{-3}$) of reference protein markers are indicated; at right the M_r values and identities of the apolipoproteins are noted. (B), apoB; (A-I), apoA-I, (A-II), apoA-II. Staining with Coomassie Blue G-250; 15 μg protein/lane. These electrophoretic patterns correspond to lipoprotein subfractions of the profile presented in Fig. 4 (male, insert B).

TABLE 1. Age-related, sex-related, and seasonal changes in the concentrations and mean weight percent of plasma lipoprotein classes in trout

		VTG		HDL		LDL		VLDL		ΣLP
Group[a]	Sex	mg/dl	wt%	mg/dl	wt%	mg/dl	wt%	mg/dl	wt%	mg/dl
A	M			1363 ± 103	77.4	260 ± 56	14.8	137 ± 4	7.8	1760 ± 130
	F	n.d.		1213 ± 118	74.4	311 ± 63	19.0	107 ± 35	6.6	1631 ± 216
B	M			1973 ± 34	54.2	510 ± 104	14.0	1158 ± 136[f]	31.8	3640 ± 57[b]
	F	1271 ± 131	28.4	2086 ± 105	46.6	332 ± 69	7.4	788 ± 57[c]	17.6	4477 ± 88[d]
C	M			2497 ± 264[d]	81.4	375 ± 99	12.2	197 ± 43	6.4	3069 ± 350
	F	1188 ± 175	28.6	2357 ± 236	56.8	393 ± 163	9.5	211 ± 26	5.1	4149 ± 190[c]
D	M			2154 ± 297[b]	74.6	534 ± 138	18.5	200 ± 40	6.9	2888 ± 454
	F	n.d.		1591 ± 210	49.0	1066 ± 186[b]	32.9	586 ± 107[b]	18.1	3243 ± 296[b]
E	M			1423 ± 49	43.9	1229 ± 79[c]	37.9	589 ± 51[c]	18.2	3241 ± 168[b]
	F	149 ± 9	3.8	1713 ± 128	44.1	1472 ± 105[c]	37.9	552 ± 4[b]	14.2	3886 ± 214[c]

Values are means ± SEM for four males (M) and three females (F). These animals were sexually nonprecocious and entered into spermiation or into ovulation at the age of 3 years. Fractionation of whole plasma lipoprotein classes and proteins was carried out by a density gradient ultracentrifugation procedure. The lipoprotein concentrations obtained in each density gradient fraction were summed between the density intervals defining the different classes of lipoproteins in trout. Density regions used were d < 1.015 g/ml for VLDL; d 1.015–1.085 g/ml for LDL = IDL + LDL; d 1.085–1.210 g/ml for HDL; d 1.210–1.310 g/ml for VTG. The concentrations were calculated as follows: total lipoproteins = cholesteryl esters + free cholesterol + triacylglycerol + phospholipids + proteins. For the calculation of VTG concentration, see Materials and Methods. The lipoprotein concentration was expressed in milligram lipoprotein per deciliter of plasma. For each group, male or female, and for each lipoprotein class, data were tested for statistical significance by ANOVA, followed by an evaluation using the Tukey-Kramer's multiple range test; n.d., not detectable by the quantitation method.

[a]A: 25 months old, December; B: 32 months old, July, for males and 33 months old, August, for females; C: 36 months old, November, for females and 37 months old, December, for males; D: 39 months old, February; E: 42 months old, May.

[b]Significant difference from the value observed for the same sex at 25 months old, $P < 0.05$.

[c]Significant difference from the value observed for the same sex at 25 months old, $P < 0.01$.

[d]Significant difference from the value observed for the same sex at 25 months old, $P < 0.005$.

[e]Significant difference from the value observed for the same sex at 25 months old, $P < 0.001$.

[f]Significant difference from the value observed for the same sex at 25 months old, $P < 0.0001$.

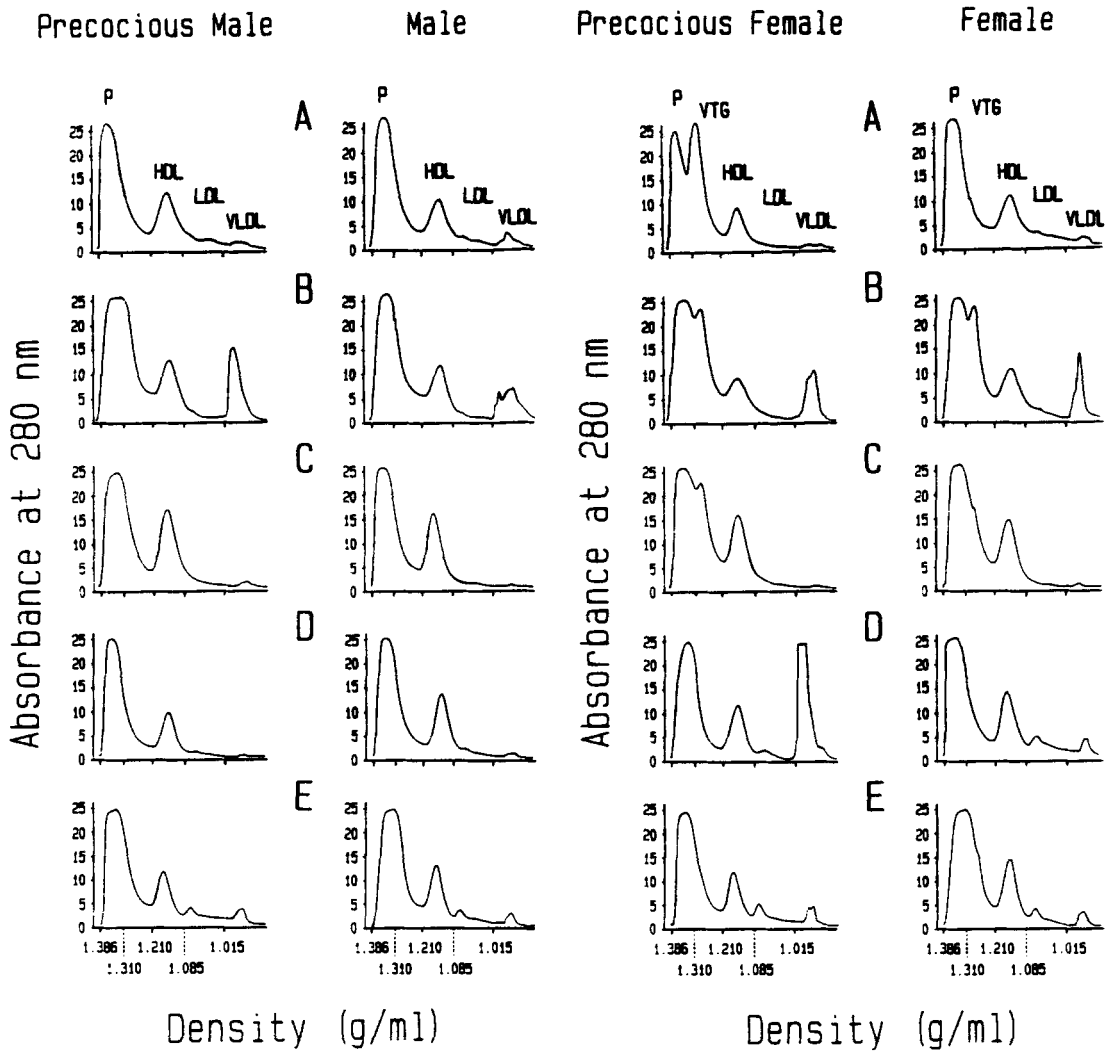

Fig. 4. Effects of age, sex, and season on plasma lipoprotein profile in trout. Ultracentrifugal single-spin absorbance profiles of plasma were obtained from the same sexually precocious male, male, precocious female, or female at different ages and seasons. A, 25 months old, December; B, 32 months old, July, for males and 33 months old, August, for females; C, 36 months old, November, for females and 37 months old, December, for males; D, 39 months old, February; E, 42 months old, May. Abscissa is the density of successive fractions from bottom of tube. One arbitrary unit of absorbance is the optical density of a 1 mg/ml solution of bovine serum albumin. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; VTG, vitellogenin; P, plasma proteins. See Table 1 for data about the lipoprotein concentrations of sexually nonprecocious animals.

precocious animals and at 2 years in the other animals, changes in lipoprotein metabolism related to the reproductive cycle were superimposed on those due to aging or to the season. The HDL-cholesterol concentration increased progressively during sexual maturation in both males and females, reaching considerable values during spermiation and at the moment of ovulation (**Fig. 5** and Table 1). A progressive decrease in the HDL-cholesterol concentration was then observed, reaching concentrations comparable to those observed in juvenile trout. During the first reproductive cycle in precocious males, the HDL-cholesterol concentration increased progressively starting in June at the age of 19 months until the onset of spermiation in December at the age of 25 months (127.4 ± 10.5 mg cholesterol/dl vs. 217.1 ± 17.6 mg cholesterol/dl). This concentration again increased during the second reproductive cycle reaching a maximum during spermiation in December at the age of 37 months (356.1 ± 44.6 mg cholesterol/dl, $P < 0.0001$). The HDL-cholesterol concentration then decreased, reaching a minimum after spermiation in the month of April at the age of 41 months (181.6 ± 25.3 mg cholesterol/dl). In males and females starting their first reproductive cycle at the age of 2 years, the HDL-cholesterol concentration remained stable until the age of 25 months (Fig. 5) and then increased progressively, reaching a maximum in males at the moment of spermiation in December, at the age of 37 months (134 ± 28.9 mg cholesterol/dl vs. 368.5 ± 38.8 mg cho-

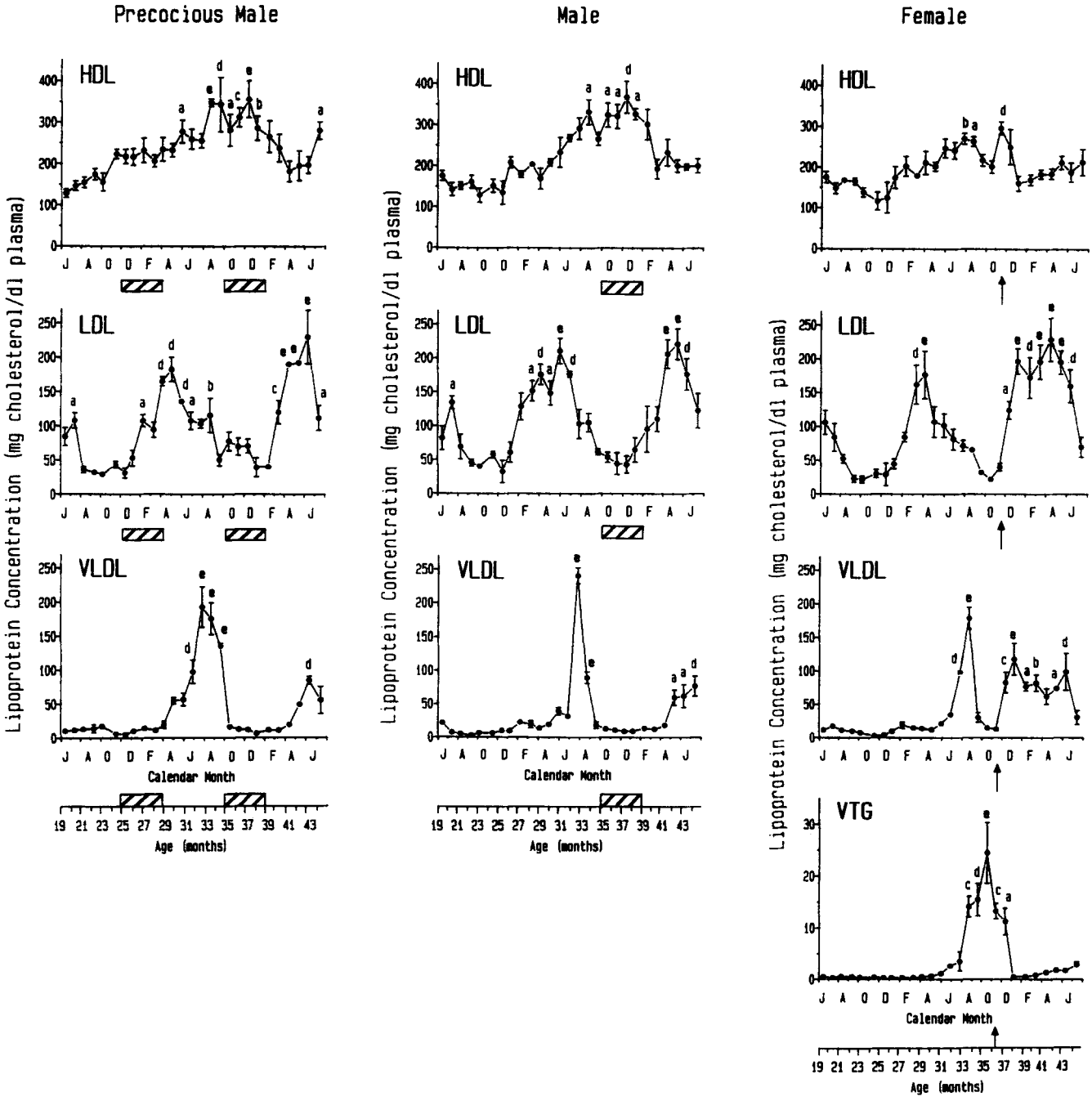

Fig. 5. Changes in the concentration of the different plasma lipoprotein classes related, to age, season, and to the reproductive cycle in trout. Every 4 weeks blood was sampled from the same group of individualized trout from the age of 19 months in June 1991 until the age of 44 months in July 1993. Three groups of animals were distinguished according to sex and the age of the first sexual maturation. The first group (precocious male) was composed of precocious males who entered into spermiation at the age of 2 years. The second group (male) was composed of males who entered into spermiation at the age of 3 years. The third group (female) was composed of females ovulating at the age of 3 years. The hatched rectangle indicates the period of spermiation and the arrow indicates the moment of ovulation. Fractionation of whole plasma lipoprotein classes and proteins was carried out by a density gradient ultracentrifugation procedure. The lipoprotein concentrations obtained in each density gradient fraction were summed between the density intervals defining the different classes of lipoproteins in trout. Density regions used were d < 1.015 g/ml for very low density lipoproteins (VLDL); d 1.015–1.085 g/ml for low density lipoproteins (LDL) = intermediate density lipoproteins + LDL; d 1.085–1.210 g/ml for high density lipoproteins (HDL); d 1.210–1.310 g/ml for vitellogenin (VTG). The lipoprotein concentration was expressed in milligram lipoprotein-cholesterol per deciliter of plasma. Values are means ± SEM for four precocious males, four males, and five females (SEM within data points are not shown). Data were tested for statistical significance by ANOVA followed by an evaluation using the Tukey-Kramer's multiple range test. For HDL, VLDL, and VTG, the significance of the differences between the first mean observed at 19 months old (first point from the left) and those observed subsequently is indicated. For LDL, the significance of the differences between the mean observed at 25 months old (the seventh point from the left) and the others is indicated; $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.005$; $^{d}P < 0.001$; $^{e}P < 0.0001$.

lesterol/dl, $P < 0.0001$). The maximum was reached in females at the moment of ovulation in November, at the age of 36 months (124.2 ± 37.9 mg cholesterol/dl vs. 295.1 ± 15.8 mg cholesterol/dl, $P < 0.0001$). These concentrations then decreased as in precocious males, reaching a minimum in March at the age of 40 months in males (193.4 ± 24.4 mg cholesterol/dl) and in January in females at the age of 38 months, (159.5 ± 18.3 mg cholesterol/dl). The HDL concentration thus practically doubled between the juvenile stage at the age of 25 months and the ovulation of females at the age of 36 months or full spermiation of males at the age of 37 months. The HDL concentration obtained is about 2500 mg/dl plasma (Table 1). This increase in the HDL concentration related to sexual maturation was confirmed by comparing the lipoprotein profiles shown in Fig. 4A and 4C.

The study of the plasma LDL-cholesterol concentration between June 1991 and July 1993 indicated a circannual variation of this concentration in precocious males, males and females (Fig. 5). These seasonal fluctuations of the LDL-cholesterol concentration were independent of animal age or their stage of sexual maturity. This circannual change in the LDL-cholesterol concentration was similar in precocious males and males. In both types of males, minimal concentrations were observed in December or January and maximal concentrations from May to July. For example, in males, between December at the age of 25 months and May at the age of 30 months, the LDL-cholesterol concentration is increased by a factor of 6.6 (32 ± 16.6 mg cholesterol/dl vs. 210.3 ± 19 mg cholesterol/dl, $P < 0.0001$). Minimal concentrations in females were observed in October and maximal concentrations in April. For example, between October at the age of 23 months and April at the age of 29 months, the LDL-cholesterol concentration in females increased by a factor of 8.4 (21 ± 5.2 mg cholesterol/dl vs. 175.9 ± 35.5 mg cholesterol/dl, $P < 0.0001$). A progressive increase in the maximal value of the LDL-cholesterol concentration obtained at each annual cycle occurred. For example, in precocious males the value obtained in July at the age of 20 months (107.6 ± 11.5 mg cholesterol/dl) was lower than the value observed in May at the age of 30 months (182.3 ± 17.7 mg cholesterol/dl) and that observed in June at the age of 43 months (229.6 ± 39.2 mg cholesterol/dl). The comparison of lipoprotein profiles between November and May in females, between 36 and 42 months of age, or between December and May in males between 37 and 42 months of age, enables the appearance of a highly pronounced LDL peak to be seen in May, while it was very low during the preceding winter (Fig. 4C and 4E). These considerable seasonal fluctuations of the plasma LDL concentration were confirmed by the quantitative values presented in Table 1.

The VLDL-cholesterol concentration after 7 days of fasting in precocious males, males and females remained relatively constant around the mean value of 11.4 ± 0.94 mg cholesterol/dl between the ages of 19 months in June and 29 months in April (Fig. 5). The concentration in April was 19.7 ± 5.4 mg cholesterol/dl in precocious males, 13.9 ± 0.8 mg cholesterol/dl in males, and 13.6 ± 2.8 mg cholesterol/dl in females. Starting at this time, the VLDL-cholesterol concentration increased considerably, reaching a maximum value in July in precocious males (192.3 ± 29.9 mg cholesterol/dl, $P < 0.0001$) and in males (240.9 ± 11.8 mg cholesterol/dl, $P < 0.0001$) and in August in females (178.9 ± 16.1 mg cholesterol/dl, $P < 0.0001$). The comparison of the lipoprotein profiles of Fig. 4A and 4B illustrates the considerable increase in the VLDL concentration related to the reproductive cycle in both sexes. The VLDL-cholesterol concentration again became minimal in precocious males and males at the onset of spermiation in October and at the moment of ovulation in females in November. The VLDL-cholesterol concentration was very high in precocious males during the 7 months separating the two spermiations and in males during the 4 months preceding the spermiation. It remained low throughout spermiation and up to the month of April, when the trout were 41 months old. Starting at this time, there was a highly significant increase in the plasma concentration VLDL-cholesterol in precocious males and in males, identical to that observed during the previous annual reproductive cycle. In females, the VLDL-cholesterol concentration increased rapidly after ovulation from November to January (12.9 ± 3.7 mg cholesterol/dl vs. 117.5 ± 23.9 mg cholesterol/dl, $P < 0.0001$). The VLDL-cholesterol concentration then decreased progressively until April and increased again as during the preceding annual reproductive cycle. During the reproductive cycle, the VLDL concentration increased by a factor of 8.4 between the age of 25 months in December and 32 months in July in males, and by a factor of 7.3 between the age of 25 months in December and 33 months in August in females (Table 1). The increased concentration of VLDL-cholesterol after the ovulation of females in November, which did not occur in spermiating males, resulted in a significant difference in the concentration of this lipoprotein class between the two sexes (200 ± 40 mg/dl in males vs. 586 ± 107 mg/dl for females, $P < 0.05$) (Table 1). This difference in the plasma VLDL levels between the two sexes is clearly illustrated by the comparison of lipoprotein profiles in Fig. 4D.

The presence of an additional peak in the lipoprotein profile in the density interval 1.210–1.310 g/ml characterizes the presence of VTG in the plasma of females during vitellogenesis (Fig. 4). The VTG-cholesterol concentration increased abruptly in July at the age of 32 months reaching a maximum in October at the age of 35 months (3.5 ± 1.8 mg cholesterol/dl vs. 24.4 ± 5.9 mg cholesterol/dl, $P < 0.0001$) (Fig. 5). At that time, the VTG concentration obtained was 2235 ± 482 mg/dl and was ob-

served 1 month before ovulation and 2 months after the maximum in the VLDL concentration. The plasma VTG-cholesterol concentration became very low in January at the age of 38 months, then increased again during the following reproductive cycle, starting in July, when the animals were 44 months old. During the reproductive cycle of females, the concentrations of VLDL, LDL, and HDL were minimal when the VTG concentration was maximal (Fig. 5).

The circannual variations of the LDL concentration or the changes in the concentration of other lipoprotein classes, VLDL, HDL, and VTG related to the annual reproductive cycle caused variations in the percentage of each lipoprotein class compared to total circulating lipoprotein concentration. This led to a significant increase in the lipoproteinemia starting at the age of 25 months (Table 1).

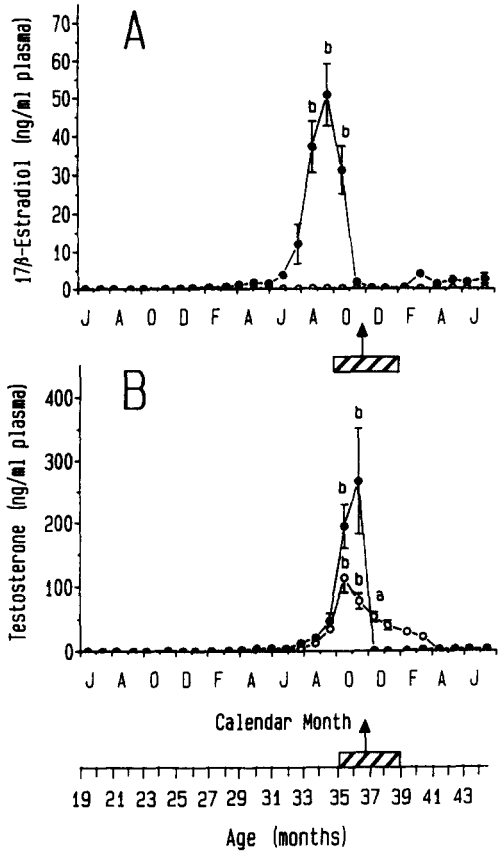

Fig. 6. Changes in plasma 17β-estradiol (A) and testosterone (B) levels in trout between the ages of 19 and 44 months. Data are presented as means ± SEM for four females (●) and four males (○). SEM or values too small for scale are not shown. Hatched rectangle indicates the spermiation period for males and the arrow indicates the time of ovulation for females. For each group, the data were tested for statistical significance by ANOVA followed by an evaluation using the Tukey-Kramer's multiple range test. The significance of the differences between the first mean observed at 19 months old (first point from the left) and those observed subsequently is indicated; [a]$P < 0.01$; [b]$P < 0.0001$.

TABLE 2. Correlation coefficients between plasma LDL-cholesterol concentration (mg/dl) and photoperiod (number of light hours per day) or water temperature (°C) in trout

Trout	LDL vs. Photoperiod[a]	LDL vs. Temperature	Photoperiod vs. Temperature
			0.839[b]
Precocious males	0.722[b]	0.402	
Males[c]	0.750[b]	0.356	
Females	0.126	−0.253	

[a]Linear regression analysis was performed for each group on 21 means determined every 4 weeks from December 1991 to July 1993.
[b]The slope was significantly different than zero at $P < 0.0001$ (two-tailed).
[c]The following regression equation was obtained: LDL-cholesterol (mg/dl) = 155.6 × number of light hours per day − 721.8.

Variations in plasma 17β-estradiol and testosterone concentrations

Changes in the plasma concentration of 17β-estradiol and testosterone in both males and females are shown in **Fig. 6** between the ages of 19 and 44 months. The 17β-estradiol concentration remained very low throughout growth and during the annual reproductive cycle in males, with a mean concentration about 37 ± 7 pg/ml. The 17β-estradiol concentration increased considerably during the reproductive cycle in females, starting in June at the age of 31 months (3.71 ± 0.66 ng/ml) and reaching a maximum in September at the age of 34 months (50.83 ± 8.34 ng/ml, $P < 0.0001$) i.e., about 2 months before the time of ovulation. The testosterone concentration increased very significantly in males throughout spermiation and reached the maximum in October at the age of 35 months (113.67 ± 22.85 ng/ml, $P < 0.0001$). This concentration then decreased progressively and became low after spermiation. The testosterone concentration in females increased significantly at the end of vitellogenesis and reached the highest value at the moment of ovulation (265.82 ± 84.97 ng/ml, $P < 0.0001$). This value is higher than the maximal concentration observed in males during spermiation.

Correlation between lipoprotein concentrations, photoperiod, and plasma steroid levels

Seasonal fluctuations in the LDL concentration were highly correlated with changes in the photoperiod in precocious males and males but not in females (**Table 2**). However, these fluctuations were not significantly correlated with changes in water temperature, in spite of the high correlation existing between the photoperiod and water temperature (Fig. 1 and Table 2). Regression analysis showed the existence of a highly significant correlation between the HDL-cholesterol concentration and the logarithm of the

testosterone concentration in males (**Fig. 7**) and in females ($r = 0.579$, $P < 0.002$). A significant correlation was also observed in females between the VTG-cholesterol concentration and the logarithm of the 17β-estradiol concentration ($r = 0.477$, $P < 0.03$).

DISCUSSION

The concentration of circulating lipoproteins is under the complex control of genetic and environmental factors. Numerous studies in mammals (see ref. 37 for review), and also in birds (38, 39), have shown that there are variations in the concentration of the different plasma lipoprotein classes during development or growth. Large, spontaneous seasonal lipid and lipoprotein changes have been documented in certain mammals such as the badger or the hedgehog, whose physiological status and endocrine activity undergo seasonal variations (40, 41). A large number of studies in humans have also shown that seasonal variations in plasma lipid and lipoprotein concentrations exist but their origin is poorly defined (1, 3–5). Trout may represent a good alternative model in order to discriminate between intrinsic and environmental factors contributing to the quantitative variations of the different lipoprotein classes in a vertebrate species. The nature and the organization of the plasma lipid transport system in trout resemble that of mammals (see ref. 7 for review). Large seasonal quantitative variations of lipids and lipoproteins may be suspected from data available before the present work and unlike in mammals it is possible to minimize genetic variability by obtaining several hundred offspring of a single male and female trout.

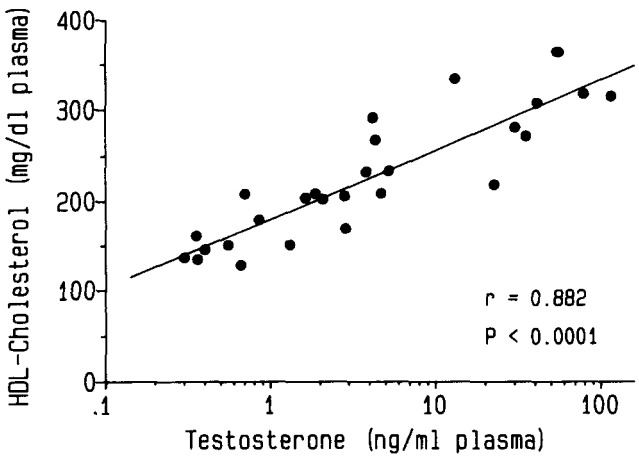

Fig. 7. Correlation between the plasma HDL-cholesterol concentration and the logarithm of the plasma testosterone concentration in male trout. Linear regression analysis was performed on 27 means determined every 4 weeks between the ages of 19 and 44 months. The following regression equation was obtained: HDL-cholesterol (mg/dl) = 76.6 log testosterone (ng/ml) + 179.4.

This report demonstrates a circannual variation in the LDL concentration in trout plasma. Minimal LDL concentrations of the order of 250 mg/dl were observed in October in females and in December or January in males. Maximal values of the order of 1300 mg/dl were reached during the month of April in females and in May to July in males. Our results also show that the annual LDL cycle exists independently of the variations in the concentration of the different lipoprotein classes observed during the reproductive cycle. This is supported by the similarity of the rhythms observed before 2 years old and after in both sexually nonprecocious male and female trout or by comparison of the LDL cycle between sexually precocious and nonprecocious males. The circannual variation in the LDL concentration results in the concomitant increase and decrease in the LDL quantitative importance in comparison to total lipoproteinemia. This would partially explain the discrepancy existing between data that indicated the predominance of LDL (10, 20) or HDL (14, 30) in the lipoprotein profile of juvenile and immature adult trout. Our study indicates that in trout the lipoprotein profile is dominated by HDL as early as the first months of life. However, a genetic variability in the plasma HDL concentration might be present as revealed by the different HDL mean concentrations reported in juvenile trout after fractionating the different lipoprotein classes by ultracentrifugation (14, 20, 30, this study).

Seasonal variations in the LDL concentration are correlated in males with the annual photocycle but not with water temperature variations. Circannual variations in the LDL concentration in males and females could be controlled by an endogenous biological clock which may be coupled with the external synchronizer (zeitgeber) which is the circannual change of the photoperiod. The presence of this endogenous circannual rhythm has been demonstrated in female trout and enables sexual maturation and the moment of ovulation to be controlled (22). Additional studies are needed for the demonstration of a self-sustained circannual LDL cycle. Experiments with constant environmental conditions would demonstrate whether the annual photocycle functions as a zeitgeber or a driver. The secretion of melatonin by the pineal gland could potentially be involved in mediating the effects of photoperiod on metabolic functions in teleosts (42–44). Sexual maturation itself could affect the plasma concentration of LDL in females. For example, the number of hepatic LDL receptors could change during the metabolic reorganization of the liver following the massive synthesis of VTG. The effect of sexual maturation would thus be superimposed on the effect of the endogenous biological clock on the LDL concentration. This could explain the phase shift of the annual cycle of the LDL concentration variation in females which would no longer be correlated with the photoperiod.

Plasma lipemia and lipoproteinemia in trout increase

progressively as a function of animal age. These augmentations result from the gradual increase in the maximum LDL concentration at each annual cycle, as well as from the considerable modifications in the levels of the different lipoprotein classes related to the annual reproductive cycle. A similar increase with age in plasma total cholesterol and LDL-cholesterol levels has been demonstrated in humans and several mechanisms have been proposed (see refs. 45–47 for reviews).

Sexual maturation in trout is accompanied by major physiological changes and leads to the formation of testicles and ovaries that may represent 6% and 15–20%, respectively, of total body weight (18, 19, 48, 49). Lipid stores represent major energy reserves in fish and, during sexual maturation in both male and female salmonids, there is a selective mobilization of lipid deposits previously stored in the perivisceral adipose tissue (19, 49, 50) or in muscles (50–53). Gonad development requires considerable supplies of constituents such as phospholipids for membranes, cholesterol as substrate for steroid production (35, 54), and lipids whose essential fatty acids are stored in oocyte yolk. The changes in the concentrations of the different lipoprotein classes observed in trout and related to phases of storage and mobilization of body lipid reserves reflect the involvement of metabolic pathways specific to the reproduction process and which involve plasma lipoproteins.

The plasma VLDL concentration after 7 days of fasting increases considerably in both sexes during the phase of lipid reserves deposit preceding the rapid increase in the gonadosomatic ratio. This increase in the VLDL concentration has been observed during the first and the second reproductive cycle in nonprecocious males and females. The maximal VLDL concentration observed was in the order of 800 mg/dl in females and 1100 mg/dl in males. This increase in the VLDL concentration during vitellogenesis is in agreement with results previously reported for female trout (17, 18). Vitellogenesis can be subdivided into an endogenous vitellogenesis corresponding to a slow ovarian growth leading to ovaries whose weight accounts for about 1% of total body weight and an exogenous vitellogenesis corresponding to a rapid ovarian growth (55, 56). In trout ovulating in November, as in this study, endogenous vitellogenesis extends from March to June and exogenous vitellogenesis from July to October. The increase in VLDL concentration observed in female trout occurs during or at the end of endogenous vitellogenesis. This is to be correlated with the very large quantity of lipids stored in perivisceral adipose tissue (19, 49, 50) during this period, resulting from the considerable increase in lipoprotein lipase and salt-resistant lipase activities in adipose tissue (19). Trout may enter into the reproductive cycle during the second year of life when sufficient body energy reserves have been accumulated during growth (22). The absence of intense storage of body lipid reserves in precocious males during the months preceding the first spermiation could explain the concomitant absence of an increase in VLDL concentration. On the other hand, after the first spermiation of precocious males and up to the onset of the following spermiation, the reconstitution of body lipid reserves results in a considerable increase in the plasma VLDL concentration. Spermiation extends over several months in male trout during which a limited ovulation period occurs in females. As a consequence, during restoration of lipid stores after ovulation, a significant difference in the VLDL concentration exists between the two sexes. The increase in VLDL concentration observed during the phase of lipid reserve deposit could be due to an increase in the level of hepatic secretion.

The catabolism and production of lipoproteins may be affected by the concentration of plasma hormones, in particular steroid hormones. In the rainbow trout, the concentration of steroid hormones, estrogens or androgens, fluctuates during the reproductive cycle (56–60). The concentration of 17β-estradiol increases considerably during exogenous vitellogenesis and induces the hepatic synthesis of VTG, a lipoprotein containing 18% of lipids of which two-thirds are phospholipids (28). Our results indicate that the VTG concentration increases abruptly in July and reaches a maximum of about 2200 mg/dl in October, i.e., 1 month before ovulation and 2 months after the maximum concentration of VLDL. During the reproductive cycle in females, the VTG-cholesterol concentration is significantly correlated with 17β-estradiol concentration and, as previously described (19), is higher when the other lipoprotein classes concentration is lowest, and vice versa. The administration of 17β-estradiol to juvenile trout induces a significant increase in the plasma concentration and hepatic secretion of VLDL, and the appearance of VTG in the plasma, independently of the nutritional status of the animals (14). The increase in plasma VLDL concentration during the reproductive cycle in females is not, however, related to the increased concentration of 17β-estradiol. In fact, the chemical and apolipoprotein compositions of the VLDL of juvenile estrogenized trout are modified (14). This is not the case for VLDL which are present in high concentration in the plasma during the reproductive cycle of females. In addition, the increase in the VLDL concentration during the reproductive cycle is observed in both males and females and there is no significant correlation in females between VLDL and 17β-estradiol concentrations. During exogenous vitellogenesis, VLDL synthesized by the liver under the effect of 17β-estradiol are very actively catabolized by growing ovaries. In addition to VTG uptake, the ovary can apparently take up lipids as a result of the lipolysis of circulating lipoproteins. This is shown by a considerable increase in lipoprotein lipase and salt-resistant lipase activities in trout ovaries during exogenous vitello-

genesis (19). The high plasma VLDL concentration observed in juvenile estrogenized trout could be due to their accumulation in the plasma resulting from the absence of ovarian lipolysis. Lipoprotein lipase in trout is considerably activated by VLDL and to a lesser extent by HDL (61).

The HDL concentration increases progressively in the course of sexual maturation, reaching considerable values on the order of 2500 mg/dl during spermiation or at the moment of ovulation. These concentrations then decrease, becoming comparable to those observed in juvenile animals. The HDL concentration in trout is highly significantly correlated with the major plasma androgen concentration, i.e., testosterone, in both males and females. The administration of testosterone to juvenile trout leads to an increase in the plasma cholesterol concentration (62). Additional work is required in order to elucidate the relationship existing in trout between the plasma HDL concentration and that of testosterone.

Modifications in the composition of plasma lipoproteins in salmonids related to the reproductive cycle (11, 63) or to seasonal fluctuations of water temperature (64, 65) have been identified. It has previously been shown that the percentage of lipids and proteins in the different lipoprotein classes was identical between juvenile male trout and those in spermiation (20). In the course of this work, no change in the percentage of lipids and proteins in the different lipoprotein classes related to age, season, or to the reproductive cycle has been identified. This does not, however, exclude the existence of this type of variation within lipoprotein subfractions. An analysis of lipoprotein subfractions, in particular at the times of minimal and maximal concentrations found in this work, is thus necessary.

In summary, the present study has shown that season and reproductive cycle are the two main factors affecting basal lipid and lipoprotein levels in trout. Independent of age and sexual maturity, a circannual variation in the LDL concentration has been demonstrated in both sexes. These seasonal fluctuations might be controlled by an endogenous biological clock able to exert an independent effect on plasma lipid and lipoprotein levels. We have also found that reproductive cycle dramatically alters the plasma concentration of VLDL and HDL in both sexes and the appearance of VTG in the plasma of females. Environmental factors such as photoperiod or endocrine factors such as the concentration of steroid hormones can be correlated and/or involved in the regulation of these quantitative variations.

Manuscript received 2 December 1993 and in revised form 28 March 1994.

REFERENCES

1. Gordon, D. J., D. C. Trost, J. Hyde, F. S. Whaley, P. J. Hannan, D. R. Jacobs, and L. G. Ekelund. 1987. Seasonal cholesterol cycles: the Lipid Research Clinics coronary primary prevention trial placebo group. *Circulation.* **76:** 1224–1231.
2. Jones, P. J. H., and D. A. Schoeller. 1990. Evidence for diurnal periodicity in human cholesterol synthesis. *J. Lipid Res.* **31:** 667–673.
3. Robinson, D., E. A. Bevan, S. Hinohara, and T. Takahashi. 1992. Seasonal variation in serum cholesterol levels—evidence from the UK and Japan. *Atherosclerosis.* **95:** 15–24.
4. Kristal-Boneh, E., G. Harari, and M. S. Green. 1993. Circannual variations in blood cholesterol levels. *Chronobiol. Int.* **10:** 37–41.
5. Mänttäri, M., K. Javela, P. Koshinen, J. Pikkarainen, V. Manninen, J. K. Huttunen, and M. H. Frick. 1993. Seasonal variation in high density lipoprotein cholesterol. *Atherosclerosis.* **100:** 257–265.
6. Grundy, S. M., and M. A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **31:** 1149–1172.
7. Babin, P. J., and J. M. Vernier. 1989. Plasma lipoproteins in fish. *J. Lipid Res.* **30:** 467–490.
8. Skinner, E. R., and A. Rogie. 1978. The isolation and partial characterization of the serum lipoproteins and apolipoproteins of the rainbow trout. *Biochem. J.* **173:** 507–520.
9. Chapman, M. J., S. Goldstein, G. L. Mills, and C. Leger. 1978. Distribution and characterization of the serum lipoproteins and their apoproteins in the rainbow trout (*Salmo gairdneri*). *Biochemistry.* **17:** 4455–4464.
10. Fremont, L., C. Leger, and M. Boudon. 1981. Fatty acid composition of lipids in the trout. II. Fractionation and analysis of plasma lipoproteins. *Comp. Biochem. Physiol.* **69B:** 107–113.
11. Babin, P. J. 1987. Apolipoproteins and the association of egg yolk proteins with plasma high density lipoproteins after ovulation and follicular atresia in the rainbow trout (*Salmo garirdneri*). *J. Biol. Chem.* **262:** 4290–4296.
12. Babin, P. J. 1987. Plasma lipoprotein and apolipoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *Biochem. J.* **246:** 425–429.
13. Larsson, A., and R. Fange. 1977. Cholesterol and free fatty acids (FFA) in the blood of marine fish. *Comp. Biochem. Physiol.* **57B:** 191–196.
14. Wallaert, C., and P. J. Babin. 1992. Effects of 17β-estradiol and starvation on trout plasma lipoproteins. *Lipids.* **27:** 1032–1041.
15. Ng, T. B., and D. R. Idler. 1983. Yolk formation and differentiation in teleost fishes. *In* Fish Physiology. Vol. IXA. W. S. Hoar, D. J. Randall, and E. M. Donaldson, editors. Academic Press, New York and London. 373–404.
16. Selman, K., and R. A. Wallace. 1989. Cellular aspects of oocyte growth in teleosts. *Zool. Sci.* **6:** 211–231.
17. Fremont, L., C. Leger, B. Petridou, and M. T. Gozzelino. 1984. Effects of a (n-3) polyunsaturated fatty acid-deficient diet on profiles of serum vitellogenin and lipoprotein in vitellogenic trout (*Salmo gairdneri*). *Lipids.* **19:** 522–528.
18. Riazi, A., and L. Fremont. 1988. Serum vitellogenin and

yolk proteolipid complex composition in relation to ovarian growth in rainbow trout *Salmo gairdneri* (Rich). *Comp. Biochem. Physiol.* **89B:** 525–529.
19. Black, D., and E. R. Skinner. 1987. 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.
20. Fremont, L., and D. Marion. 1982. A comparison of the lipoprotein profiles in male trout (*Salmo gairdneri*) before maturity and during spermiation. *Comp. Biochem. Physiol.* **73B:** 849–855.
21. Farner, D. S. 1985. Annual rhythms. *Annu. Rev. Physiol.* **47:** 65–82.
22. Duston, J., and N. Bromage. 1988. The entrainment and gating of the endogenous circannual rhythm of reproduction in the female rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol. A.* **164:** 259–268.
23. Wallaert, C., and P. J. Babin. 1994. Effects of temperature variations on dietary lipid absorption and plasma lipoprotein concentrations in trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* In press.
24. Nichols, D. J. 1987. Fluid volumes in rainbow trout, *Salmo gardneri:* application of compartmental analysis. *Comp. Biochem. Physiol.* **87A:** 703–709.
25. Gingerich, W. H., and R. A. Pityer. 1989. Comparison of whole plasma body and tissue blood volumes in rainbow trout (*Salmo gairdneri*) with ^{125}I bovine serum albumine and ^{51}Cr-erythrocyte tracers. *Fish Physiol. Biochem.* **6:** 39–47.
26. Wallaert, C., and P. J. Babin. 1994. Frozen storage affects high density lipoproteins and the quantitation of trout (*Oncorhynchus mykiss*) lipoprotein classes as separated by ultracentrifugation. *Comp. Biochem. Physiol.* In press.
27. Wallace, R. A. 1985. Vitellogenesis and oocyte growth in nonmammalian vertebrates. *In* Developmental Biology, a Comprehensive Synthesis. Vol. 1. L. W. Browder, editor. Plenum Press, New York and London. 147–177.
28. Norberg, B., and C. Haux. 1985. Induction, isolation and a characterization of the lipid content of plasma vitellogenin from two *Salmo* species: rainbow trout (*Salmo gairdneri*) and sea trout (*Salmo trutta*). *Comp. Biochem. Physiol.* **81B:** 869–876.
29. Ayrault-Jarrier, M., J. Burdin, L. Fremont, and M. T. Gozzelino. 1988. Immunological evidence for common antigenic sites in high-density lipoproteins from rainbow trout and man. *Biochem. J.* **254:** 927–930.
30. Babin, P. J. 1992. Binding of thyroxine and 3,5,3'-triiodothyronine to trout plasma lipoproteins. *Am. J. Physiol.* **262:** E712–E720.
31. Delcuve, G. P., J. M. Sun, and J. R. Davie. 1992. Expression of rainbow trout apolipoprotein A-I genes in liver and hepatocellular carcinoma. *J. Lipid Res.* **33:** 251–262.
32. Neuhoff, V., N. Arold, D. Taube, and W. Ehrhardt. 1988. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis.* **9:** 255–262.
33. Belcher, J. D., J. O. Egan, G. Bridgman, R. Baker, and J. M. Flack. 1991. A micro-enzymatic method to measure cholesterol and triglyceride in lipoprotein subfractions separated by density gradient ultracentrifugation from 200 microliters of plasma or serum. *J. Lipid Res.* **32:** 359–370.
34. Markwell, M. A. K., S. M. Hass, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
35. Babin, P. J. 1986. Effect of plasma lipoproteins in gonadotropin stimulation of 17β-estradiol production in the ovarian follicle of rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocrinol.* **64:** 456–467.
36. SAS Institute Inc. 1988. SAS Procedure Guide, Release 6.03. SAS Institute Inc., Cary, North Carolina.
37. Chapman, M. J. 1986. Comparative analysis of mammalian lipoproteins. *Methods Enzymol.* **128:** 70–143.
38. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure and comparative aspects. *J. Lipid Res.* **21:** 789–853.
39. Tarugi, P., D. Reggiani, E. Ottaviani, S. Ferrari, R. Tiozzo, and S. Calandra. 1989. Plasma lipoproteins, tissue cholesterol overload, and skeletal muscle apolipoprotein A-I synthesis in the developing chick. *J. Lipid Res.* **30:** 9–22.
40. Laplaud, P. M., L. Beaubatie, and D. Maurel. 1982. Further characterization of the changes occurring in the plasma lipoprotein spectrum in the European badger (*Meles meles L.*) during winter. *Biochim. Biophys. Acta.* **711:** 213–223.
41. Laplaud, P. M., Saboureau, L. Beaubatie, and B. El-Omari. 1989. Seasonal variations of plasma lipids and lipoproteins in the hedgehog, an animal model for lipoprotein[a] metabolism: relation to plasma thyroxine and testosterone levels. *Biochim. Biophys. Acta.* **1005:** 143–156.
42. Delahunty, G., G. Bauer, M. Prack, and V. De Vlaming. 1978. Effects of pinealectomy and melatonin treatment on liver and plasma metabolites in the goldfish, *Carassius auratus. Gen. Comp. Endocrinol.* **35:** 99–109.
43. Gern, W. A., and S. S. Greenhouse. 1988. Examination of in vitro melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. *Gen. Comp. Endocrinol.* **71:** 163–174.
44. Binkley, S. 1993. Structures and molecules involved in generation and regulation of biological rhythms in vertebrates and invertebrates. *Experientia.* **49:** 648–653.
45. Spady, D. K., and J. M. Dietschy. 1989. Interaction of aging and dietary fat in the regulation of low density lipoprotein transport in the hamster. *J. Lipid Res.* **30:** 559–569.
46. Ericsson, S., M. Eriksson, S. Vitols, K. Einarsson, L. Berglund, and B. Angelin. 1991. Influence of age on the metabolism of plasma low density lipoproteins in healthy males. *J. Clin. Invest.* **87:** 591–596.
47. Bertolotti, M., N. Abate, S. Bertolotti, P. Loria, M. Concari, R. Messora, F. Carubbi, A. Pinetti, and N. Carulli. 1993. Effect of aging on cholesterol 7α-hydroxylation in humans. *J. Lipid Res.* **34:** 1001–1007.
48. Billard, R. 1987. The reproductive cycle of male and female brown trout (*Salmo trutta fario*): a quantitative study. *Reprod. Nutr. Dévelop.* **27:** 29–44.
49. Nomura, M. 1963. Studies on reproduction of rainbow trout, *Salmo gairdneri,* with special reference to egg taking—V. Development of gonads and size of fish spawned firstly. *Bull. Jpn. Soc. Sci. Fish.* **29:** 976–984.
50. Nassour, I., and C. L. Leger. 1989. Deposition and mobilization of body fat during sexual maturation in female trout (*Salmo gairdneri*). *Aquat. Living Resour.* **2:** 153–159.
51. Tveranger, B. 1985. Variation in growth rate, liver weight and body composition at first sexual maturity in rainbow trout. *Aquaculture.* **49:** 89–99.
52. Aksnes, A., B. Gjerde, and S. O. Roald. 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon (*Salmo salar*). *Aquaculture.* **53:** 7–20.
53. Phleger, C. F., Laub, R. J., and A. A., Benson. 1989. Skeletal lipid depletion in spawning salmon. *Lipids.* **24:** 286–289.

54. Loir, M. 1990. Trout steroidogenic testicular cells in primary culture. I. Changes in free and conjugated androgen and progestagen secretions: effects of gonadotropin, serum, and lipoproteins. *Gen. Comp. Endocrinol.* **78:** 374–387.
55. Van Bohemen, C. G., J. G. D. Lambert, and J. Peute. 1981. Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.* **44:** 94–107.
56. Van Bohemen, C. G., and J. G. D. Lambert. 1981. Estrogen synthesis in relation to estrone, estradiol, and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.* **45:** 105–114.
57. Scott, A. P., and J. P. Sumpter. 1983. A comparison of the female reproductive cycles of autumn-spawning and winter-spawning strains of rainbow trout (*Salmo gairdneri* Richardson). *Gen. Comp. Endocrinol.* **52:** 79–85.
58. Schulz, R. 1984. Serum levels of 11-oxotestosterone in male and 17β-estradiol in female rainbow trout (*Salmo gairdnei*) during the first reproductive cycle. *Gen. Comp. Endocrinol.* **56:** 111–120.
59. Baynes, S. M., and A. P. Scott. 1985. Seasonal variations in parameters of milt production and in plasma concentration of sex steroids of male rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocrinol.* **57:** 150–160.
60. Scott, A. P., and J. P. Sumpter. 1989. Seasonal variations in testicular germ cell stages and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. *Gen. Comp. Endocrinol.* **73:** 46–58.
61. Skinner, E. R., and A. M. Youssef. 1982. The characterization of lipoprotein lipase isolated from the post-heparin plasma of the rainbow trout, *Salmo gairdneri* Richardson. *Biochem. J.* **203:** 727–734.
62. Bunde, C. J. W., and E. W. House. 1993. Induction of spawning levels of cortisol or testosterone in juvenile trout, *Oncorhynchus mykiss* (Walbaum). *Comp. Biochem. Physiol.* **105A:** 431–436.
63. Ando, S., and M. Hatano. 1988. Isolation of apolipoproteins from carotenoid-carrying lipoprotein in the serum of chum salmon, *Oncorhynchus keta. J. Lipid Res.* **29:** 1264–1271.
64. Wallaert, C., and P. J. Babin. 1993. Circannual variation in the fatty acid composition of high-density lipoprotein phospholipids during acclimatization in trout. *Biochim. Biophys. Acta.* **1210:** 23–26.
65. Wallaert, C., and P. J. Babin. 1994. Thermal adaptation affects the fatty acid composition of plasma phospholipids in trout. *Lipids.* **29:** 373–376.