Transport of n–3 fatty acids from the intestine to the retina in rats

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Abstract This study was undertaken to determine the mode of transport of the essential (n–3) fatty acids docosahexaenoic acid 22:6(n–3) and linolenic acid 18:3(n–3). Male weanling Sprague-Dawley rats received a mixture of corn oil and [^{14}C]18:3(n–3) or [^{14}C]22:6(n–3) by gavage. At periods of 1 to 4 days after the injection, four rats per time point were killed and samples of blood were taken via heart puncture and the livers and retinas were collected. Blood lipoproteins and plasma proteins were separated by ultracentrifugation and analyzed by HPLC. Lipids were extracted and saponified and the fatty acids were converted to phenacyl esters for separation of individual fatty acids. After 1 and 2 h, radioactivity from 18:3(n–3) and 22:6(n–3) was observed primarily in the chylomicron/very low density lipoprotein fraction. By 4 h, radioactivity in the lipoprotein fraction was greatly decreased, with a small amount of radioactivity associated with albumin in the soluble protein fraction. After 24 h, the total amount of radioactivity associated with lipoprotein was further reduced, with more than half of the remaining label occurring in association with albumin and another unidentified protein. In the liver, 22:6(n–3) was concentrated in triacylglycerols (40.7%) and phospholipids (51.1%), with a maximum specific activity at 4 h. In the rod outer segments (ROS), the specific activity of [^{14}C]22:6(n–3) increased to a maximum at 24 h and maintained a high level even at 4 days. These data suggest that after injection, 18:3(n–3) and 22:6(n–3) are esterified to triglyceride and phospholipid by the intestinal absorptive cells and transported in chylomicrons to the liver. After conversion of 18:3(n–3) to 22:6(n–3) in the liver, the retina accumulates 22:6(n–3) which may be transported from the liver via albumin and another unidentified protein, and is retained by the rod outer segments.—**Li, J., M. Wetzel, and P. J. O'Brien.** Transport of n–3 fatty acids from the intestine to the retina in rats. *J. Lipid Res.* 1992. **33:** 539–548.

Supplementary key words albumin • chylomicrons • docosahexaenoic acid • linolenic acid • lipoprotein • photoreceptor • retinal debris • rod outer segments • very low density lipoprotein

Docosahexaenoic acid (22:6(n–3)) is an essential polyunsaturated fatty acid containing 22 carbons and 6 double bonds. While a minor constituent of blood and most tissues, 22:6(n–3) is found in high concentrations in the phospholipids of brain synaptosomes, retina, and sperm (1). The photoreceptor outer segments of vertebrate retinas contain the highest concentration of 22:6(n–3) which comprises up to one-half of the fatty acids of phosphatidylethanolamine (PE) and phosphatidylserine (PS) and somewhat smaller amounts of phosphatidylcholine (PC) in the disc phospholipids (2, 3). Although the precise role of 22:6(n–3) is not understood, the retina is known to retain this fatty acid tenaciously during dietary insufficiency of n–3 fatty acids, and oxidation of this highly unsaturated fatty acid may cause photoreceptor damage (2).

While much is known about the conversion of linolenic acid (18:3(n–3)) to 22:6(n–3) (4, 5) and the distribution of 22:6(n–3) in phospholipids (6), little has been learned about the uptake of 22:6(n–3) and its major dietary precursor 18:3(n–3) in the intestine and their subsequent distribution to tissues via the blood. The present investigation describes the distribution of radioactivity in blood plasma, liver, and retinas of young weanling rats fed corn oil containing [^{14}C]18:3(n–3) or [^{14}C]22:6(n–3). The distribution of n–3 fatty acids in various substituents of the blood plasma including lipoproteins and soluble proteins was of particular interest. Scott and Bazan (7) have previously described the conversion of 18:3(n–3) to 22:6(n–3) in the liver after intraperitoneal injection of isotope, with subsequent distribution of 22:6(n–3) to the brain and retina. This route of entry of isotope bypasses the intestinal absorptive cells which normally esterify long chain fatty acids to triglycerides and subsequently package them into chylomicrons for distribution via the lymphatics and blood (8). The current study demonstrates that dietary 18:3(n–3) and 22:6(n–3) are transported in the blood in a manner similar to that of

Abbreviations: HPLC, high performance liquid chromatography; ROS, rod outer segments; RD, retinal debris; TLC, thin-layer chromatography; FAPE, fatty acid phenacyl esters; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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other long chain fatty acids, suggesting that target tissues such as brain and retina probably contain specific mechanisms such as receptors and fatty acid-binding proteins for accumulating and retaining essential fatty acids.

MATERIALS AND METHODS

Experimental animals

Male weanling Sprague-Dawley rats (125 g) were obtained from Taconic Farms (Germantown, NY). They were fed standard lab chow and maintained on a 12-h light and 12-h dark cycle. [1-^{14}C]18:3(n–3) (24 μCi/mmol) and [1-^{14}C]22:6(n–3) (24 μCi/mmol), obtained from New England Nuclear, Boston, MA, were evaporated to dryness under nitrogen and the fatty acids were dissolved in small volumes of ethanol. Rats were fasted overnight and then 3 μCi ^{14}C-labeled fatty acid per rat in corn oil (0.3 ml) was introduced into the stomach by gavage. Four rats were used for each timepoint, two receiving a mixture of corn oil and [^{14}C]18:3(n–3) and two receiving a mixture of corn oil and [^{14}C]22:6(n–3). At periods of 1, 2, and 4 h and 1, 2, 3, and 4 days after the gavage, animals were killed, 4–5 ml of blood per rat was obtained via heart puncture, and samples of livers and whole retinas were collected. Crude rod outer segments (ROS) and the remaining retinal debris (RD) were prepared by sucrose density gradient centrifugation as described previously (5).

Plasma preparation

Blood was collected in Vacutainers with 0.15 ml of a solution containing 30 mg of EDTA as anticoagulant (Vacutainer, Becton-Dickinson & Co., Rutherford, NJ). Blood samples were immediately centrifuged at 4°C in a refrigerated centrifuge at 1500 *g* for 20 min to sediment cells, and plasma was removed with a plastic pipette.

Initial centrifugation of lipoprotein

One ml of plasma per rat was raised to 1.225 g/ml by addition of solid potassium bromide (J.T. Baker Chemical Co.) (0.3517 g KBr/ml of plasma) and placed in the bottom of a 28.3-ml polyallomer tube. The plasma was carefully overlayered with a solution of 1.225 g/ml (adding solid KBr to 0.9% NaCl), according to Rudel et al. (9). The tubes were placed in a Beckman SW 41 TI rotor and centrifuged at 40,000 rpm for 48 h at 15°C. The lipoprotein migrated through a threefold volume of KBr to the top of the tube. Lipoproteins (the cloudy layer at the top of tube) (0.8 to 1 ml) and the lipoprotein-free plasma proteins at the bottom of the tube) (2–3 ml) were removed with a Pasteur pipette and stored at 4°C.

HPLC analysis of plasma samples

Samples (0.2 ml) of whole plasma, lipoproteins, and lipoprotein-free plasma proteins, respectively, were analyzed using a Spherogel TSK 4000 SW gel permeation column, 7.5 mm × 30 cm (Beckman Instruments, Inc.) with an LKB Bromma HPLC system. Each sample was eluted with 0.1 M Tris-HCl buffer (pH 7.4) at a flow rate of 1 ml/min (10). Protein peaks were detected by UV monitoring at 275–285 nm. Individual classes of lipoproteins and lipoprotein-free plasma proteins were separated; the peaks were collected and radioactivity was counted in Ready-Gel (Beckman Instruments, Inc.) using a Beckman 2800 scintillation counter. Bovine serum albumin and hemoglobin standards were run to identify known serum components. The patterns of eluted lipoproteins matched those of Christie (11).

Lipid extraction and separation

Lipids from liver and retina were separated as previously described (11). A 1-ml aliquot of fresh rat lipoprotein or lipoprotein-free plasma protein from each peak fraction separated by HPLC was extracted with chloroform–methanol (12), and the extract was stored in a freezer under nitrogen.

Lipid analysis

Neutral lipids were separated from phospholipids by one-dimensional thin-layer chromatography (1D-TLC) using 20 × 20 cm, 250 μm Silica Gel H plates (Analtech) and a solvent system of hexane–ethyl ether–acetic acid 80:20:2. Individual phospholipids were separated by two-dimensional thin-layer chromatography (2D-TLC) according to the method of Anderson, Maisde, and Feldman (13). Aliquots of each sample were taken for total radioactivity and phosphorus determination (14) prior to chromatography. Lipid spots were visualized with iodine vapors and autoradiograms were prepared for all thin-layer plates using EN^{3}Hance spray (New England Nuclear, Boston, MA) and Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY). Spots were then scraped and the radioactivity was counted.

Preparation of fatty acid phenacyl esters (FAPE)

FAPE were prepared according of the method of Hanis et al. (15) to visualize the fatty acids under UV light. Extracted lipids were taken to dryness under nitrogen and immediately suspended in 1 ml of 3.3% KOH in ethanol. Samples were saponified for 40 min at 55°C, then 1 ml of water was added. The samples

were acidified to pH 2.0 with 50 µl concentrated HCl. The free fatty acids were extracted with 3 ml of hexane. The hexane phase was dried under nitrogen and resuspended in 50 µl of acetone containing 10 mg/ml of bromoacetophenone (Sigma) and mixed with 50 µl of acetone containing 10 mg/ml of triethylamine (Sigma). The sample was tightly capped and heated in a boiling water bath for 5 min. The sample was cooled and 70 µl of acetone containing 2 mg/ml of acetic acid was added. The sample was taken to dryness with nitrogen and resuspended in 100 µl of acetonitrile.

HPLC analysis of FAPE

The fatty acid phenacyl esters were separated by HPLC using a 25 cm × 4.6 mm LC-18 reverse phase column (Supelco Inc.) (16). Each 20-µl sample was eluted with a gradient from acetonitrile–water 80:20 (HPLC grade, Beckman) to acetonitrile–water 90:10 over 95 min. FAPE absorption was monitored by UV absorbance at 254 nm. Radioactivity was measured with a Beckman 171 Radioisotope Detector using Ready-Solv HP (Beckman) with a ratio of one part mobile phase to three parts scintillation cocktail at a flow rate of 1.5 ml/min. Samples were also collected and radioactivity was measured with a Beckman LS 2400 scintillation counter. The purity of the radiolabeled fatty acids used was 96% for 18:3(n–3) and 97% for 22:6(n–3) as determined by the HPLC of the phenacyl esters.

Other methods

Protein concentrations were determined by the method of Bradford (17). Fatty acid standards were purchased from the following sources: linolenic acid, oleic acid, docosahexaenoic acid, arachidonic acid from Sigma (St. Louis, MO); palmitic acid, and stearic acid from Calbiochem (San Diego, CA). All fatty acids standards were of analytical-reagent grade. The LC-18 column was calibrated with phenacyl derivatives of the fatty acid standards.

RESULTS

Young male rats that had been fasted overnight were fed a mixture of corn oil and [^{14}C]22:6(n–3) or [^{14}C]18:3(n–3) by gavage. Four separate experiments were conducted with 22:6(n–3) with essentially identical results; and ten experiments were conducted with 18:3(n–3), likewise with essentially identical results. **Fig. 1** illustrates the separation of protein-containing components achieved with this methodology as measured by absorption at 280 nm. Whole plasma contained proteins from the lipoprotein and lipoprotein-free plasma protein fractions with the latter representing the major constituents. Albumin was the most prominent plasma protein peak with immunoglobin and fibrinogen peaks eluting ahead of albumin. The hemoglobin peak just following albumin was partially obscured by albumin and varied with the degree of hemolysis of erythrocytes in each sample. In the lipoprotein fraction, the chylomicron/VLDL peak was distinct from other peaks and varied in size with the dietary state of the animal, being very low during fasting and high for a few hours after ingestion of lipid. Only the lipid-rich chylomicron/VLDL, low density lipoprotein (LDL) and cholesterol-rich high density lipoprotein (HDL_2) were of sufficient buoyancy to float to the top of the KBr layer. The less lipid-enriched HDL species (HDL_3) remained at the bottom of the tube with the remaining plasma proteins. In rats, LDL constitutes a very minor fraction of the total lipoprotein.

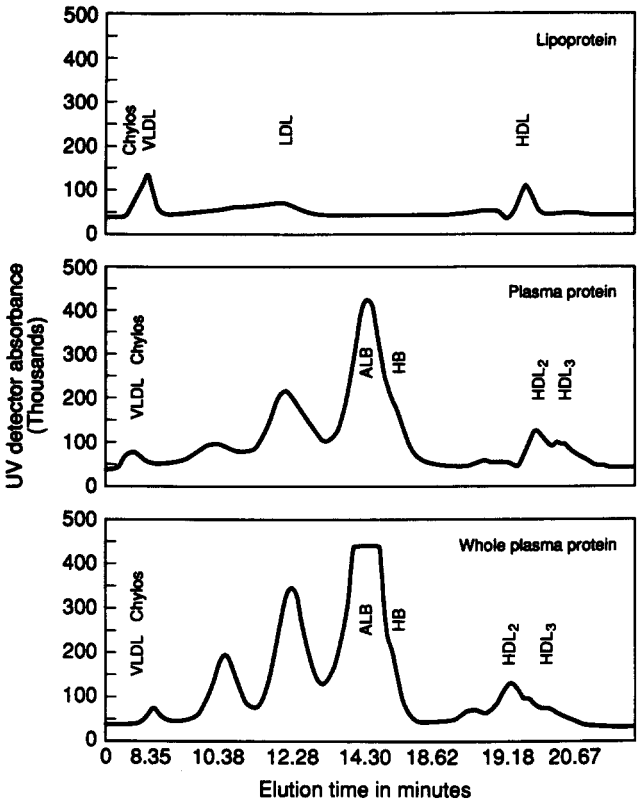

Fig. 1. HPLC fractions of rat lipoprotein, lipoprotein-free plasma protein, and whole plasma protein fractions were monitored by absorbance at 275–285 nm for protein. Samples were eluted with 0.1 M Tris-HCl buffer (pH 7.4) from a Spherogel TSK 4000 SW gel permeation column. The major components are: very low density lipoproteins/chylomicrons (VLDL/Chylos), high density lipoprotein, (HDL), albumin (Alb), hemoglobin (HB), high density lipoprotein-2 (HDL_2), and high density lipoprotein-3 (HDL_3). This elution profile is representative of ten essentially identical determinations made 24 h post-gavage.

After ingestion of [^{14}C]22:6(n–3) most of the radioactivity was associated with the lipoprotein fraction for

the first 24 h. A similar distribution was noted for 18:3(n–3). When either [^{14}C]18:3(n–3) or [^{14}C]22:6 (n–3) was fed to rats, a single peak of radioactivity appeared in the whole plasma at 1 and 2 h post-ingestion. At early times, this radioactivity was associated with the chylomicron/VLDL peak of the lipoprotein fraction (**Fig. 2**). The radioactivity in this fraction was associated primarily (92%) with triglycerides when analyzed by thin-layer chromatography. At later times, the radioactivity of the chylomicron/VLDL peak decreased and a second more diffuse peak of radioactivity, probably representing several components including chylomicron remnants, VLDL, intermediate density lipoprotein (IDL), and LDL components, was noted at 4 and 24 h and continued to be detectable for several days. A smaller but distinct peak was also present coincident with the albumin peak in whole plasma and in the lipoprotein-free plasma protein fraction at 1, 2, 4, and 24 h after ingestion of either n–3 fatty acid (**Fig. 3**). Rats that were fed [^{14}C]22:6(n–3) also showed a second small peak of radioactivity eluting slightly ahead of albumin at 4, 24, and 48 h which may represent a second fatty acid-binding protein.

A summary of the radioactivity of lipid classes in livers of rats after ingestion of [^{14}C]22:6(n–3) is shown in **Fig. 4**. A similar profile was observed when [^{14}C]18:3(n–3) was ingested. Neutral lipids and phospholipids were extracted and separated by thin-layer chromatography. Triglycerides were the predominant labeled species at 1 and 2 h, while at later times phospholipids were more heavily labeled. Initially, the intestinal absorptive cells esterified dietary n–3 fatty acids primarily to triglycerides as they do with other long chain fatty acids. These triglycerides were then probably packaged into chylomicrons which were released into the lymph and subsequently appeared in the circulating blood. The liver took up the radiolabeled triglyceride either by the action of hepatic

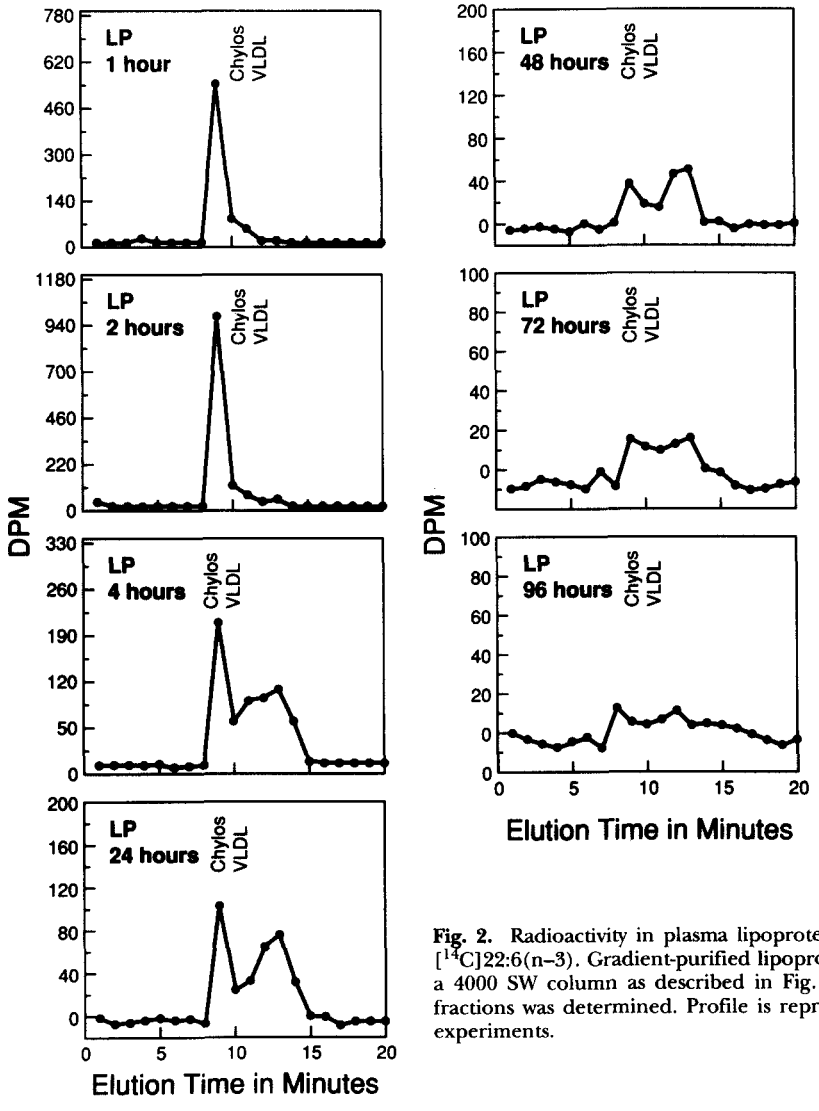

Fig. 2. Radioactivity in plasma lipoproteins (LP) after gavage with [^{14}C]22:6(n–3). Gradient-purified lipoproteins were fractionated on a 4000 SW column as described in Fig. 1 and radioactivity in the fractions was determined. Profile is representative of four separate experiments.

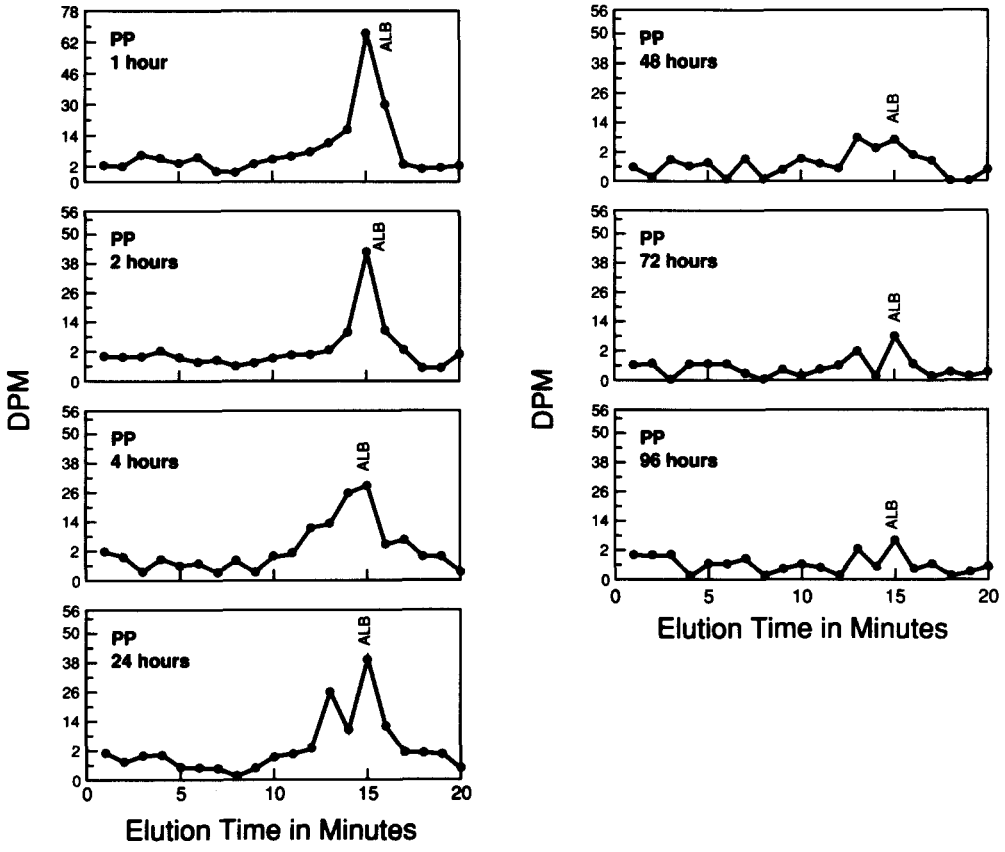

Fig. 3. Radioactivity in lipoprotein-free plasma protein fractions (PP) from density gradients of rat plasma after gavage with [^{14}C]22:6(n–3). The plasma proteins were eluted from a Spherogel TSK 4000 SW column. Fractions were collected and the radioactivity was measured. Data are expressed as dpm per sample. The profile is representative of four separate experiments.

lipase on chylomicrons and/or the phagocytic uptake of chylomicron remnants via the apoE receptor. At later times, some of this radioactivity appeared in phospholipids, some possibly associated with synthesis of VLDL constituents in the liver. The ester bonds linking radiolabeled n–3 fatty acids to chylomicron triglycerides had therefore been cleaved by lipolytic enzymes and the fatty acids reesterified to phospholipids in the liver.

After gavage with [^{14}C]22:6(n–3), the total specific activity of lipids increased in the liver from 1 to 4 h after ingestion. By 24 h, the specific activity had decreased to less than one-half that noted at 4 h and radiolabeled lipids continued to decrease steadily over the next 3 days. Phenacyl esters of fatty acids from the total liver lipid fraction were separated by HPLC and most of the radioactivity was associated with the peak of 22:6(n–3) at 2 and 4 h after ingestion of [^{14}C]22:6(n–3) (**Fig. 5**). A peak of the retroconversion product 22:6(n–3) was also noted in the liver when [^{14}C]22:6(n–3) was ingested, particularly in the first few hours post-ingestion. In addition, small amounts of 16:0, 18:0, and 18:1 were present in the liver due to metabolic breakdown of 22:6(n–3) and de novo synthesis of these nonessential fatty acids.

The transport of n–3 fatty acids from the intestine to the retina is via the circulatory system. However, while the liver accumulated radioactivity relatively soon after [^{14}C]22:6(n–3) ingestion with the highest specific activity noted at the 4-h time point (Figs. 4 and 5), specific activity in the retina was not maximal until 24 h after ingestion (**Fig. 6**). By 24 h, the specific activity of liver has already dropped to one-half its value at 4 h. This discrepancy in the accumulation of radiolabeled 22:6(n–3) between liver and retina suggests that the retina may not take up 22:6(n–3) directly from chylomicrons but rather from either VLDL synthesized in the liver or from fatty acids released from the liver bound to albumin or other transport proteins. The liver has been shown by Scott and Bazan (7) to be a major site for conversion of 18:3(n–3) to 22:6(n–3). The present data indicate that dietary 22:6(n–3) may also be routed through the liver to the retina.

Fig. 6 illustrates the time course of labeling in individual fatty acids associated with plasma and retina after [^{14}C]22:6(n–3) ingestion. Most of the ad-

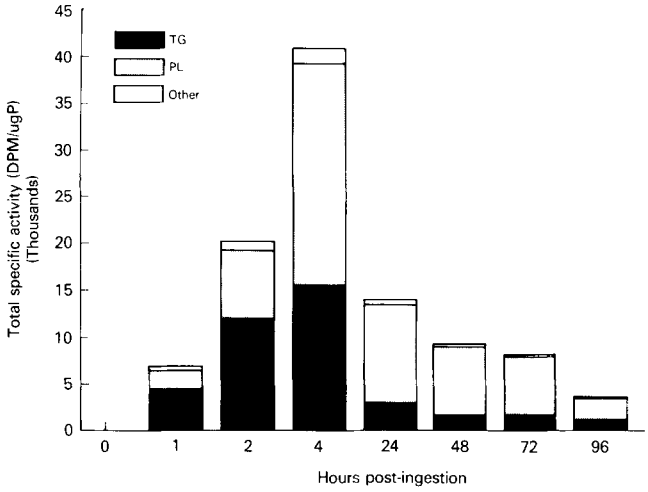

Fig. 4. Specific activity of individual classes of liver lipids after gavage with [^{14}C]22:6(n–3). Total extracted lipids were separated by 1D-TLC and visualized with autoradiography prior to counting. PL, phospholipids; TG, triglycerides; Other, includes diglycerides and unesterified fatty acids. Data are expressed as dpm/μg of organic phosphorus in the lipid extract and are representative of four separate experiments.

ministered isotope remained as 22:6(n–3) in the chylomicron/VLDL fraction and in the retina while both radiolabeled 22:6(n–3) and palmitate (16:0) were associated with albumin in plasma, presumably due to the metabolic breakdown of 22:6(n–3) and de novo synthesis of fatty acids in the liver (see Fig. 5). A small amount of radiolabeled saturated and monounsaturated fatty acids was also present in the retina after ingestion of [^{14}C]22:6(n–3). Labeling of 22:6(n–3) in the ROS increased rapidly and peaked at 24 h, whereas the labeling in the retinal debris (RD) increased steadily for 4 days at a much lower specific activity. However, the radioactive 22:6(n–3) in the ROS fraction varied from 96% to 92% of the total label in the retina from the first to the fourth day.

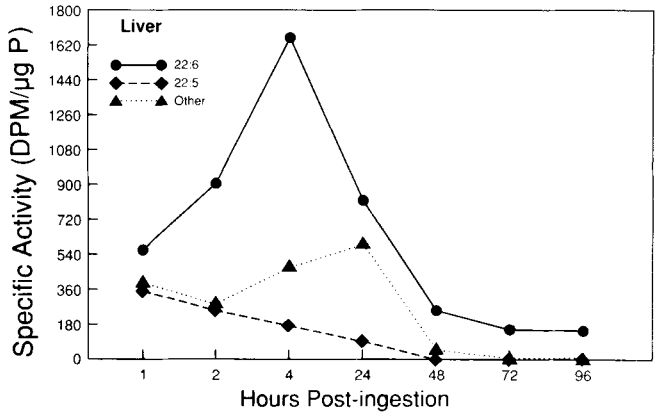

Fig. 5. Time course of ^{14}C radioactivity associated with individual polyunsaturated fatty acids in the total liver lipid fraction after gavage with [^{14}C]22:6(n–3). Lipids were saponified and the fatty acids were esterified. HPLC fractions were monitored for fatty acid phenacyl esters and radioactivity as described in Methods. Data are expressed as dpm/μg phosphorus in the lipid extract and are representative of four separate experiments.

The profile of radiolabeled fatty acids was strikingly different when 18:3(n–3) was ingested. **Fig. 7** shows that while 18:3(n–3) was the major radiolabeled fatty acid in chylomicrons/VLDL at 1–2 h after ingestion, the liver rapidly converted 18:3(n–3) to 22:6(n–3) and several intermediates in the elongation and desaturation pathway to 22:6(n–3), mainly 20:5(n–3) and 22:5(n–3), while a smaller amount was metabolized and appeared as radiolabeled 16:0. Retinal fractions accumulated very little radiolabeled 18:3(n–3) or 22:6(n–3) when [^{14}C]18:3(n–3) was ingested. A peak of radiolabeled 16:0 was noted at 24 h and decreased rapidly in the following 2 days.

DISCUSSION

The n–3 and n–6 unsaturated fatty acids are essential dietary constituents, as animal tissues are unable to synthesize double bonds at the third or sixth carbons from the methyl terminal of fatty acids. The first double bond that animals are capable of synthesizing is in the n–9 position (18). Linolenic acid (18:3(n–3)) is the most abundant dietary source of n–3 fatty acids and is found in many plant oils. Marine algae, plankton, and fish contain significant quantities of 22:6(n–3), the latter representing a major dietary source of this fatty acid for man. Animals contain enzymes capable of elongating and desaturating 18:3(n–3) to 22:6(n–3) (19). These same enzymes may also act upon fatty acids in the n–6 series modifying linoleic acid (18:2n–6) to 22:5n–6 (20). The two pathways appear to compete with one another and a dietary deficit of n–3 fatty acids results in an increase in production of n–6 fatty acids (21).

Lipids containing long-chain fatty acids are broken down in the digestive tract and the fatty acids are subsequently absorbed by the small intestine. The intestinal absorptive cells reesterify long-chain fatty acids to form triglycerides which are incorporated into chylomicrons (22). Chylomicrons are secreted into the lymph and subsequently released into the blood stream. Lipoprotein lipases in various tissues hydrolyze chylomicron and VLDL triglyceride, releasing fatty acids which enter cells. Dietary short- and medium-chain fatty acids follow a different uptake pathway that does not entail the synthesis of triglycerides and chylomicrons (23). Data from the current study show that ingested 18:3(n–3) and 22:6(n–3) are transported in the blood from the intestine primarily by chylomicrons/VLDL in a manner similar to other long-chain fatty acids. The smaller radiolabeled particles

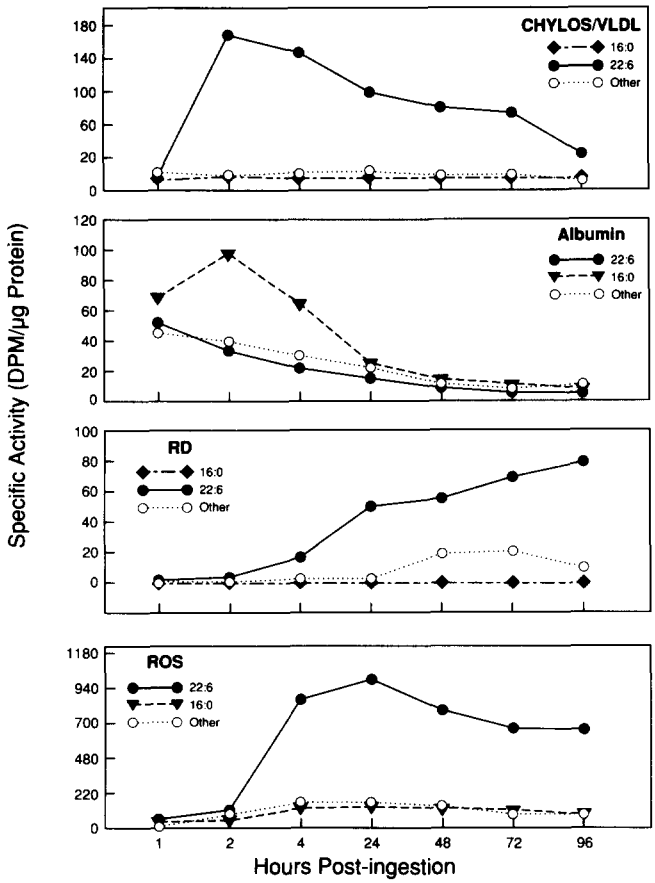

Fig. 6. Radioactivity in fatty acid phenacyl esters of chylomicrons (Chylos)/very low density lipoprotein (VLDL), albumin, retinal debris (RD), and rod outer segments (ROS), after gavage with [^{14}C]22:6(n–3). The samples were analyzed as described in Methods. Other fatty acids include short chain (<12 carbon atoms) in albumin and 18:0 in RD. Data are expressed as dpm/µg protein. Each retinal fraction contained lipid from two retinas and the data are representative of four separate experiments.

that appear at later times in the lipoprotein fraction probably represent either chylomicron remnants or VLDL and LDL produced by the liver from radiolabeled precursors delivered by chylomicrons. Several recent studies have involved feeding fish oil rich in n–3 fatty acids to rats and measuring the fatty acid content of lymph from thoracic duct cannulation (24, 25). Chernenko et al. (24) have measured a maximum of n–3 enriched triglyceride in the lymph from 90–120 min after gavage. They assumed that this triglyceride was in chylomicrons synthesized in the intestine.

Albumin is known to be the primary carrier of unesterified fatty acids in the blood plasma of adult vertebrates (see review by Spector, ref. 26). The current data show that albumin may also transport 18:3(n–3) and 22:6(n–3), but much smaller amounts of radioactivity were noted in association with albumin than with lipoproteins in the first few hours after ingestion of radiolabeled n–3 fatty acids. There was another unidentified protein (Fig. 3) that might also transport polyunsaturated fatty acids in the plasma of adult rats. The possibility remains that this may be a specific carrier, with albumin acting as a nonspecific carrier in times of excess 22:6(n–3) availability. Alpha fetoprotein is believed to serve this function in the fetus, according to Parmelee, Evenson, and Deutsch (27).

The liver appears to be an important site of elongation and desaturation of n–3 fatty acids (7, 28). Our data are in agreement with these earlier studies. In the current study, by 2 and 4 h radioactivity was high in liver after ingestion of both 18:3(n–3) and 22:6(n–3). By 24 h, total radioactivity in the liver had dropped to about one-half that noted at 4 h. There was some retroconversion of 22:6(n–3) to 22:5(n–3) in the liver as early as 1 h after ingestion as noted previously by Anderson, Connor, and Corliss (29). In rats that had ingested 18:3(n–3), some of the radioactivity in the liver at 24 h was associated with palmitate and oleate.

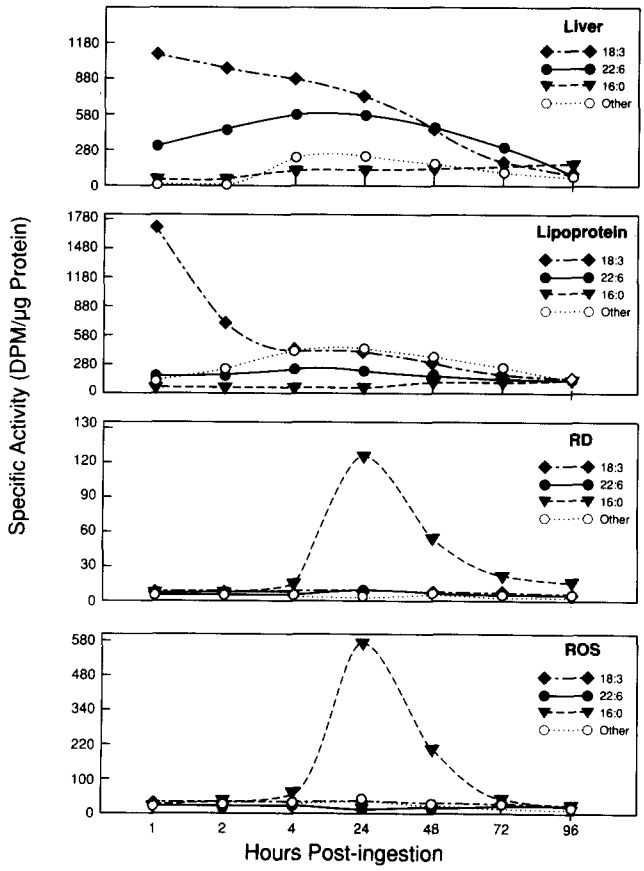

Fig. 7. Summary of ^{14}C radioactivity associated with individual fatty acid phenacyl esters after gavage with [^{14}C]18:3(n–3). Liver, lipoprotein, retinal debris (RD), and rod outer segments (ROS) samples were analyzed as described in Methods. Data are expressed as dpm/µg protein per sample containing two retinas and are representative of ten separate experiments.

This finding indicates that 18:3(n–3) had been metabolized and the metabolic products were subsequently used for de novo synthesis of these relatively saturated fatty acids as previously observed by Salem, Kim, and Yergey (30). Furthermore, it appears that most of those metabolic products were transported out of the liver with significant accumulation in the retina (Fig. 7). Similarly, Anderson and Connor (31) noted significant conversion of labeled 18:3(n–3) to brain cholesterol after its injection into rat jugular veins. Likewise, Scott and Bazan (7) observed significant conversion of intraperitoneally injected 18:3(n–3) to 16:0 in retina and brain lipids of developing rats. But, unlike the present study, they also observed long term accumulation of 22:6 in both retina and brain, probably because of the nearly hundredfold higher dose of 18:3 (n–3) used. Consistent with this interpretation was the prevalence of 18:3(n–3) as an unesterified free fatty acid in the rat livers at early times in the Scott and Bazan study (7) whereas, in the present study, 18:3(n–3) was found mainly in triglyceride as early as 1 h.

In the current study, synthesis of 22:6(n–3) by the liver was significant with the maximum amount of radiolabel at 24 h after ingestion of [^{14}C]18:3(n–3). On the other hand, there was no significant uptake of 18:3(n–3) by the retina, reinforcing the suggestion that dietary essential fatty acids are first cycled through the liver before transport to the retina. This, too, is in agreement with Scott and Bazan (7) who reported that both 18:3(n–3) and 22:6(n–3) transiently passed through the liver with little or no subsequent accumulation of 18:3(n–3) in either brain or retina. The current study differs from that of Scott and Bazan (7) in that conversion of 18:3(n–3) to 22:6(n–3) in the liver was overshadowed by catabolic degradation. As a result, very little radioactive 22:6(n–3) found its way to the retina. The higher dose used by Scott and Bazan (7) was sufficient to overcome this degradative loss. Nevertheless, the present experiments with labeled 22:6(n–3) show that this fatty acid is transported from the liver to the retina regardless of its origin as dietary 22:6(n–3) or 18:3(n–3).

A surprising observation was the large transient peak of radiolabeled 16:0 in the retina at 24 and 48 h after ingestion of [^{14}C]18:3(n–3). It is known that 16:0 is very important to photoreceptor function as it is the fatty acid involved in palmitoylation of the rod photopigment rhodopsin (32). In addition, the stored form of vitamin A in the pigment epithelial cells is primarily the palmitate ester, 16:0, serving as the preferred fatty acid for this esterification (33, 34). It seems likely from the current data that both 22:6(n–3) and 16:0 may be acquired by the retina from the liver via the plasma, probably through the choroidal circulation and pigment epithelium.

It is important to note that Anderson and Connor (31) observed rapid uptake of unsaturated fatty acids in the brains of hepatectomized developing rats. Thus, it seems clear that neural tissues are capable of obtaining these fatty acids from the circulation regardless of their origin. In the hepatectomized rats, the fatty acids were probably bound to albumin which may be an effective mode of transport that permits selective uptake to occur.

The retina contains all of the enzymes necessary for the conversion of 18:3(n–3) to 22:6(n–3), but this pathway is very slow in the retina, taking many days for completion of the slowest step, the final desaturation from 22:5(n–3) to 22:6(n–3) (5). The liver appears to play an important role in normal conversion of dietary 18:3(n–3) to 22:6(n–3). The relative importance of retina and liver in 22:6(n–3) synthesis may depend on the developmental stage of the animal as well as the nutritional state of the animal. In the present study, only well-nourished normal young rats were studied. No conclusions may therefore be drawn regarding the biological importance of the n–3 pathway in the eye over the life-span of an animal under more environmentally realistic conditions. It does seem clear, however, as noted previously by others (29), that dietary 22:6(n–3) can accumulate in the retina very rapidly and efficiently compared with an equivalent amount of dietary 18:3(n–3) converted to 22:6(n–3). The rapid uptake of 22:6(n–3) in ROS is indicative of the continuous need for this fatty acid in ROS membrane renewal (2). In contrast, the slower accumulation of 22:6(n–3) in the ROS-free retinal debris probably reflects its use in the turnover of synaptic membranes which are known to be enriched in 22:6(n–3) (35, 36).

A severe dietary deficiency of n–3 fatty acids during development has been found to cause irreversible retinal damage despite repletion with 22:6(n–3) (37). This finding emphasizes the fact that a continuous supply of 22:6(n–3) is necessary during normal development, whether directly or via conversion of 18:3(n–3). While the amount of 22:6(n–3) is normally very low in plasma, tissues that need this essential fatty acid have a very efficient system for concentrating and retaining it for long periods if necessary. It is highly likely that specific binding and/or transport proteins that play a vital role in the deployment of polyunsaturated fatty acids will be found.

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