Lipoprotein lipase expression level influences tissue clearance of chylomicron retinyl ester

Arlette M. van Bennekum,* Yuko Kako,† Peter H. Weinstock,§ Earl H. Harrison, Richard J. Deckelbaum,* Ira J. Goldberg,† and William S. Blaner[1,*,†]**

Institute of Human Nutrition* and Department of Medicine,† Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032; Laboratory of Biochemical Genetics and Metabolism,§ The Rockefeller University, New York, NY 10021; and Department of Biochemistry,** Allegheny University of the Health Sciences, Philadelphia, PA 19129

Abstract Approximately 25% of postprandial retinoid is cleared from the circulation by extrahepatic tissues. Little is known about physiologic factors important to this uptake. We hypothesized that lipoprotein lipase (LpL) contributes to extrahepatic clearance of chylomicron vitamin A. To investigate this, [^{3}H]retinyl ester-containing rat mesenteric chylomicrons were injected intravenously into induced mutant mice and nutritionally manipulated rats. The tissue sites of uptake of ^{3}H label by wild type mice and LpL-null mice overexpressing human LpL in muscle indicate that LpL expression does influence accumulation of chylomicron retinoid. Skeletal muscle from mice overexpressing human LpL accumulated 1.7- to 2.4-fold more ^{3}H label than wild type. Moreover, heart tissue from mice overexpresssing human LpL, but lacking mouse LpL, accumulated less than half of the ^{3}H-label taken up by wild type heart. Fasting and heparin injection, two factors that increase LpL activity in skeletal muscle, increased uptake of chylomicron [^{3}H] retinoid by rat skeletal muscle. Using [^{3}H]retinyl palmitate and its non-hydrolyzable analog retinyl [^{14}C]hexadecyl ether incorporated into Intralipid emulsions, the importance of retinyl ester hydrolysis in this process was assessed. We observed that ^{3}H label was taken up to a greater extent than ^{14}C label by rat skeletal muscle, suggesting that retinoid uptake requires hydrolysis. In summary, for each of our experiments, the level of lipoprotein lipase expression in skeletal muscle, heart, and/or adipose tissue influenced the amount of [^{3}H]retinoid taken up from chylomicrons and/or their remnants.—van Bennekum, A. M., Y. Kako, P. H. Weinstock, E. H. Harrison, R. J. Deckelbaum, I. J. Goldberg, and W. S. Blaner. **Lipoprotein lipase expression level influences tissue clearance of chylomicron retinyl ester. *J. Lipid Res.* 1999.** 40: **565–574.**

Supplementary key words vitamin A • retinoids • postprandial lipids • lipase • transgenic mice • lipid emulsions

Dietary retinol is taken up by the intestinal mucosa and is incorporated as retinyl ester along with other dietary lipids into chylomicrons (1–6). The nascent chylomicrons are secreted into the lymphatic system and enter the general circulation where they are quickly metabolized (5, 6). This metabolism involves both the hydrolysis of triglycerides present in the core of the particle by lipoprotein lipase (LpL) and the exchange of apolipoproteins (5, 6). As a result of these processes, a smaller particle is generated, the chylomicron remnant. The remnant particle is thought to contain most of the retinyl esters originally packaged in the nascent chylomicrons (1, 5, 6). Chylomicron remnants are rapidly cleared primarily by hepatocytes, where shortly after uptake the retinyl esters undergo hydrolysis (1, 6, 7). After hydrolysis the resulting retinol is either reesterified for storage within the liver or bound to retinol-binding protein (RBP) and secreted into the circulation for delivery to retinoid responsive tissues (1, 7, 8). Although it has long been known that some extrahepatic tissues including skeletal muscle, heart, kidney, and adipose tissue take up significant amounts of dietary retinoid (1–3), the physiological factors and processes responsible for uptake and the importance of this extrahepatic uptake are not understood.

LpL is expressed in several tissues, including skeletal muscle, heart, adipose tissue, kidney, and macrophages (5, 9–11). After secretion from its cellular sites of synthesis, LpL associates with heparan sulfate proteoglycans (HSPG) on the luminal endothelial cell surface (5, 6, 9, 12). The physiological actions of HSPG-bound LpL in lipoprotein metabolism may include both hydrolysis of core triglyceride and facilitation of uptake of lipoprotein remnant particles by cells (5, 6, 9, 12). This latter action may involve the LpL molecule serving as a bridge to link lipoproteins to cell surfaces (5, 6, 9, 13), thus, mediating lipoprotein uptake by receptors like the low density lipo-

Abbreviations: LpL, lipoprotein lipase; RBP, retinol-binding protein; HSPG, heparan sulfate proteoglycans; WT, wild type; MCK, muscle creatine kinase promoter; PBS, 10 mM Na_3PO_4, 150 mM NaCl, pH 7.4; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; and LDL, low density lipoprotein.

[1] To whom correspondence should be addressed.

protein receptor and the low density lipoprotein-related protein or uptake by non-receptor mediated pathways.

We (14) and others (15) have reported that LpL can catalyze hydrolysis of chylomicron retinyl esters after most of the triglyceride present in the chylomicrons has first been hydrolyzed (14). We further demonstrated that the amount of total retinol (retinol + retinyl ester) taken up by murine BFC-1β adipocyte cultures from retinyl ester-containing chylomicrons was greatly increased by addition of exogeneous LpL to the cultured cells (14). Thus, LpL concentration influenced cellular uptake of retinyl esters from either chylomicrons and/or their remnants. Based on these observations, we hypothesized that differences in the level of LpL expression and/or LpL activity influence tissue uptake of chylomicron retinyl esters by extrahepatic tissues in vivo (14). The present study explores this possibility.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 400–450 g (Charles River Laboratories, Wilmington, MD) were housed in a room undergoing a 12-h light–dark cycle and provided access to standard pelleted rodent chow (Ralston Purina Company, Richmond, VA) and water ad libitum. For experiments with chylomicrons, rats were either fed ad libitum or fasted for 24 h prior to the start of experiments. For experiments with Intralipid emulsions, rats were fasted only for 12 h.

Three strains of mice were used for our studies. These consisted of wild type C57BL/6J mice that express control levels of LpL (WT mice), totally LpL-deficient mice in the C57BL/6J background that overexpress human LpL in muscle (MCK/LpL0 mice), and C57BL/6J mice that overexpress human LpL in muscle (MCK/LpL2 mice). For the mouse strains overexpressing human LpL, expression was driven by the muscle creatine kinase (MCK) promoter. The generation and characteristics of these induced mutant mice have been reported previously (16). For all studies, we used 3- to 5-month-old male and female mice. Prior to all experiments, mice were fasted 12 h.

Preparation of rat chylomicrons containing [^{3}H]retinyl palmitate

Rat chylomicrons were prepared as described by Goodman et al. (2). Briefly, overnight fasted male Sprague-Dawley rats (200–300 g) were administered 0.2 mL of a 1:1 mixture of corn oil and olive oil containing 40 μCi [^{3}H]retinol ([11,12-^{3}H(N)]retinol, 37.3 Ci/mmol, DuPont NEN, Boston, MA) by gastric cannula. The oil solution also contained unlabeled retinol and α-tocopherol at a final concentration of 2 mg per 0.5 mL. The animals were anesthetized by intraperitoneal injection of a mixture of Ketaset and Xylazine (final dose: 2 mL containing 141 mg Ketaset and 12 mg Xylazine per kg rat). Within 20 min after infusion of the retinol-containing corn oil solution, the superior mesenteric lymph duct was cannulated and the milky chyle was collected into a sterile 15-mL polystyrene tube containing 0.5 mg EDTA in 0.5 mL saline (0.9% NaCl) which was maintained on ice and in the dark. Chyle production and collection continued for periods up to 24 h. After the start of chyle production, rats were provided free access to a solution of 0.9% NaCl, 0.05% KCl, and 5% glucose in water. After collection, chyle was overlayered with 0.05% EDTA in PBS (10 mm Na_3PO_4, 150 mm NaCl, pH 7.4) and centrifuged at 110,000 *g* and 18°C for 30 min to separate the chylomicrons from other substances present in the chyle. After centrifugation, the tube was sliced and the chylomicrons were aspirated. Chylomicron preparations were stored at 4°C in the dark for periods up to 4 days prior to use in our experiments.

Preparation of Intralipid emulsions doubly labeled with [^{3}H]retinyl palmitate and retinyl [^{14}C]hexadecyl ether

[^{3}H]retinyl palmitate was synthesized from all-*trans* retinol and palmitic anhydride as described previously (17). Retinyl [^{14}C]hexadecyl ether also was synthesized according to procedures reported previously (18). Briefly, [^{14}C]hexadecyl mesylate was synthesized from 100 μCi [1-^{14}C]palmitic acid (50 mCi/mmol, DuPont NEN) and the labeled mesylate was reacted with 100 mg unlabeled retinol (which had been purified immediately prior to use on a column of 10% deactivated alumina), to yield retinyl [^{14}C]hexadecyl ether. The newly synthesized radiolabeled retinoids were purified on columns of 5% deactivated alumina followed by chromatography on a normal phase HPLC system (14).

In order to generate emulsions containing both labeled retinoids, 15 μCi [^{3}H]retinyl palmitate and 10 μCi retinyl [^{14}C]hexadecyl·ether, both in hexane containing 0.01% butylated hydroxy toulene (BHT), were added to a small amber glass vial and the solvent was slowly evaporated to dryness under N_2. Immediately upon reaching dryness, 0.5 mL of a 5% solution of Intralipid (diluted in sterile PBS from a stock of 20% Intralipid, Kabi Pharmacia Inc., Clayton, NC) was added to the dry retinoids. The Intralipid solution was sonicated 3 times for 20 sec each at a power setting of 40 Watt using a Branson Sonifier Cell Disruptor (Model W185) (Branson Scientific, Inc., Plainview, NY) to bring the dried retinoids into solution. To remove the small amounts of titanium released from the sonifier probe, the retinoid-containing Intralipid emulsion was centrifuged in an Eppendorf centrifuge at 14,000 *g* for 15 min at 4°C. The resulting emulsion was stored in the dark at 4°C for up to 7 days prior to use in experiments.

To assess whether the radiolabeled retinoids had been incorporated in a similar manner and to a similar extent into the Intralipid emulsion and to demonstrate that sonication and retinoid inclusion had not disrupted the emulsion particles, small aliquots of the emulsion taken immediately after sonication and of the unsonicated emulsion were transferred into 80-μL capillary tubes and centrifuged for 10 min in a hematocrit centrifuge. After centrifugation, the tubes were cut into 7 sections (each 0.5 cm in length) and radioactivity present in each section was assessed along with the emulsion triglyceride and phospholipid concentrations present in the sections. If the composition of the emulsion had not changed and if the radiolabeled retinoids were homogeneously distributed within the emulsion, then the ratios of both radiolabels, and the triglyceride and phospholipid concentrations in each section of the capillary tube should remain constant. The ratio of triglyceride to phospholipid concentration in each centrifuged fraction obtained for control Intralipid emulsion was identical to that of the sonicated Intralipid emulsion. Therefore, neither the process of sonication nor the incorporation of the tracer doses of the radiolabeled retinoids into the emulsion had influenced the composition of the emulsion. Moreover, as greater than 90% of total ^{3}H and ^{14}C cpm placed into the capillary tube were recovered in the top fraction after centrifugation and as the ratios of ^{3}H cpm, ^{14}C cpm, triglyceride, and phospholipid remained constant, the labeled retinoids must have been incorporated homogeneously into the Intralipid emulsion.

Animal manipulations

For injection of chylomicrons containing [^{3}H]retinyl ester, rats were anesthetized, the right jugular vein was surgically ex-

posed, and 0.4 mL of a solution of rat chylomicrons containing 12 mg triglyceride and 1.7 μCi (46 nmol) [^{3}H]retinyl ester was injected into the exposed vein. For the experiments where rats received simultaneous injections of heparin with the chylomicrons, the 0.4-mL dose consisted of 0.2 mL chylomicron preparation diluted with 0.2 mL of heparin (100 IU/kg body weight) in saline.

At 5 and 10 min after injection of the [^{3}H]retinyl ester-containing chylomicrons, blood samples were taken from a lateral tail vein to assess serum clearance of the ^{3}H label. Twenty minutes after injection, the animals were bled from the descending aorta and a total body perfusion was performed using an ice-cold solution of 0.9% NaCl containing 0.5 mm EDTA at a flow rate of 4.5 mL/min for 5 min in order to remove residual blood from the tissues. Liver, spleen, epididymal fat, kidney, lung, heart, and skeletal (gastrocnemicus) muscle were excised quickly, immediately frozen in liquid N_2, and stored at −70°C prior to analysis. Blood samples were allowed to clot at 4°C for several hours and were subsequently centrifuged at 2,000 *g* for 15 min at 4°C to separate the serum from the cells. Aliquots of serum collected 5, 10, and 20 min after chylomicron injection were taken for liquid scintillation counting to assess the clearance of the labeled retinoid(s) from the circulation and another aliquot was frozen at −70°C for further use in analysis.

For injection of rats with Intralipid emulsion containing [^{3}H]retinyl ester and retinyl [^{14}C]hexadecyl ether, a two-sided jugular vein cannulation was performed 1 day prior to the start of experiments (19). The anesthesia used before and during the surgery was the same as described above for collection of chylomicrons. Rats were allowed to recover from the surgery for 12 h and during recovery they were fasted. After recovery and while fully awake, the animals were injected with a dose consisting of 0.4 mL of a 0.92% Intralipid emulsion containing 0.8 μCi (0.015 nmol) [^{3}H]retinyl palmitate and 0.15 μCi (0.57 nmol) retinyl [^{14}C]hexadecyl ether through a patent cannula in the left jugular. A dose of 4 mg of triglyceride was injected into each animal. Immediately after dose injection, aliquots of blood not exceeding 0.1 mL were taken from the opposite jugular cannula at regular time intervals in order to assess disappearance of the labels from the circulation. Animals were anesthetized, bled, and killed 20 min after injection of the dose. Tissue dissection and storage were carried out as described above for chylomicron [^{3}H]retinyl ester injections.

Fasted mice were injected through the right jugular vein or the tail vein with approximately 0.1 mL of a solution of rat chylomicrons containing 0.2 μCi (12 nmol) [^{3}H]retinyl palmitate and 3 mg triglyceride. Serum clearance of the ^{3}H label was assessed at 5 and 10 min after injection in samples obtained through tail vein bleed. Animals were bled and killed after 20 min, at which time total body perfusion was performed for 2 to 3 min as described above for the rat experiments. The liver, spleen, kidney, brain, heart, skeletal muscle, intraperitoneal, subcutaneous and epididymal fat were excised and immediately frozen in liquid nitrogen and stored at −70°C prior to analysis for [^{3}H]retinoid content.

Liquid scintillation counting

To assess levels of [^{3}H]retinoid taken up by tissues of experimental animals (rats or mice) from either radiolabeled chylomicrons or radiolabeled Intralipid emulsion, 20% (w/v) tissue homogenates were made in PBS using a Polytron Tissue Disrupter (Brinkmann Instruments, Westbury NY). Each tissue homogenate was extracted with 6 volumes of chloroform–methanol 2:1 (v/v) as described previously (19). After vigorous mixing, centrifugation to separate phases and removal of the lower lipid containing chloroform phase, the total lipid extracts were allowed to evaporate in a standard laboratory hood and the resulting lipid film was redissolved in 20 mL Hydrofluor liquid scintillation counting solution (National Diagnostics, Atlanta, Georgia). ^{3}H and ^{14}C cpm were assayed simultaneously in a Beckman LS 1800 Liquid Scintillation Counter. The spillover of ^{14}C cpm into the ^{3}H channel was 4.8% and the spillover of ^{3}H cpm into the ^{14}C channel was 7.5%. For total lipid extracts that showed color (especially the liver extracts) a quench correction was carried out by adding known amounts of ^{3}H cpm and ^{14}C cpm to the lipid extracts after initial counting in order to assess the absolute efficiency of scintillation counting for each sample. For serum clearance curves, the total serum volume for rats and mice was estimated to be equal to 2.75% of the total body weight of the animal (20).

Separation of [^{3}H]retinol and [^{3}H]retinyl esters by normal phase HPLC or by chromatography on neutral alumina

For rats that had been injected with Intralipid emulsion containing both [^{3}H]retinyl ester and retinyl [^{14}C]hexadecyl ether, the percentage of [^{3}H]retinol present in serum and intracellularly in liver, lung, and spleen was assessed by normal phase HPLC as described above. In order to determine the ratio of [^{3}H]retinol to [^{3}H]retinyl ester in serum of fed and fasted rats with or without heparin injection, serum was extracted with 20 volumes of chloroform–methanol 2:1 20 min after injection with chylomicrons containing [^{3}H]retinyl ester. The chloroform extracts were evaporated to dryness under a gentle steam of N_2 and the retinoids were redissolved in hexane and applied to a 1 × 10 cm column of 10% deactivated alumina in order to separate retinol and retinyl esters (21, 22). The [^{3}H]retinyl esters were eluted with 3% diethyl ether in hexane, after which the [^{3}H]retinol was eluted with 50% diethyl ether in hexane. The retinol- and retinyl ester-containing fractions were evaporated to dryness and redissolved in Hydrofluor for liquid scintillation counting.

Reverse phase HPLC determination of tissue retinol and retinyl ester levels

WT, MCK/LpL0, and MCK/LpL2 mouse tissues were homogenized in 2 mL PBS using a Polytron Tissue Disruptor. After homogenization, an equal volume of absolute ethanol containing internal standard retinyl acetate was added. Retinol and retinyl esters were extracted into 5 mL hexane, subjected to a backwash with 1 mL of deionized H_2O, and subsequently evaporated under a gentle stream of N_2. Immediately upon reaching dryness, the extract was redissolved in 40 μL of benzene for injection onto the HPLC. Retinol and retinyl ester (retinyl linoleate, retinyl oleate, retinyl palmitate, and retinyl stearate) levels were analyzed using a reverse-phase HPLC procedure as described previously (23) on a 4.6 × 250 mm 5μm Beckmann Ultrasphere C18 column (Beckmann Instruments, Inc.). Retinoids were separated in a mobile phase consisting of acetonitrile–methanol–dichloromethane 70:15:15 (v/v) flowing at 1.8 mL/min. The total retinol values represent the sum of retinol, retinyl linoleate, retinyl oleate, retinyl palmitate, and retinyl stearate and are expressed as μg retinol equivalents per gram wet weight of tissue.

Analysis of triglyceride and phospholipid levels

Triglyceride levels were assayed by an enzymatic procedure using a commercial kit according to the accompanying instructions (Boehringer Mannheim Diagnostics, Indianapolis, IN). Phospholipid levels were determined using a sulfuric acid digestion procedure exactly as described in the literature (24).

Statistical analysis

All data are expressed as means ± one standard deviation (SD). Statistical significance was determined by Student's un-

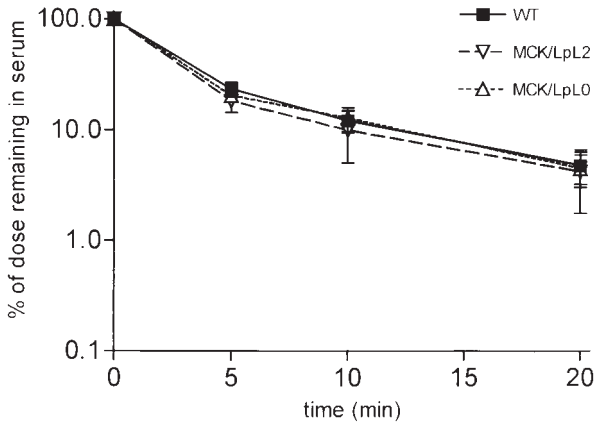

Fig. 1. The clearance of rat mesenteric chylomicrons containing [^{3}H]retinyl ester from the circulations of fasted WT (■), MCK/LpL0 (△), and MCK/LpL2 (▽) mice. Values represent the percent of the injected dose of ^{3}H cpm remaining in the circulation at each time. As described in Materials and Methods, a bolus dose consisting of 3 mg triglyceride and 0.2 μCi (12 nmol) [^{3}H]retinyl ester was injected intravenously into 4 mice of each strain. These data were obtained for one of duplicate experiments which gave essentially identical results. The error bars indicate ± 1 standard deviation. The plasma volume of each mouse was taken to be 2.75% of total body weight (20).

paired *t*-test (two-tailed). Group differences were rejected as not significant at $P > 0.05$.

RESULTS

Clearance and tissue uptake of chylomicron [^{3}H]retinyl ester in wild type and genetically modified mice

To assess whether modulation of the level of expression of LpL in tissues can alter the uptake of retinoid from chylomicrons, we studied the clearance of rat mesenteric chylomicrons containing [^{3}H]retinyl ester in three different strains of mice: wild type (WT), LpL-deficient mice that specifically overexpress human LpL in muscle only (MCK/LpL0), and wild type mice similarly overexpressing human LpL in muscle (MCK/LpL2). This experiment was carried out two independent times using 4 mice from each strain for each experiment. The two replicate experiments gave identical results; consequently, we are presenting representative data in **Fig. 1** and **Fig. 2** for one of the two experiments. Shown in Fig. 1 are the plasma clearance curves of the ^{3}H label for each of these strains of mice after intravenous injection of a bolus dose of radiolabeled chylomicrons. The rates of clearance of the label for the WT, MCK/LpL0, and MCK/LpL2 animals were not statistically different. This is in agreement with previously published investigations of these mice (25).

Although the overall recoveries of the ^{3}H label in tissues for WT, MCK/LpL0, and MCK/LpL2 mice were not statistically different for the strains and averaged respectively 59 ± 4%, 54 ± 4%, and 55 ± 3%, respectively, we did observe differences in the tissue sites of uptake of chylomicron [^{3}H]retinyl ester between strains (Fig. 2). Amongst the tissues investigated, uptake of ^{3}H label by skeletal muscle was markedly influenced by the level of LpL expression. The uptake of [^{3}H]retinoid was significantly greater for both strains of mice that express the human LpL transgene in skeletal muscle compared to WT mice. These data, expressed as ^{3}H cpm/g of tissue, are shown in Fig. 2. Uptake of ^{3}H label by skeletal muscle for MCK/LpL0 mice was 1346 ± 275 ^{3}H cpm/g (mean ± SD), for MCK/LpL2 mice 950 ± 225 ^{3}H cpm/g, and for WT mice 577 ± 138 ^{3}H cpm/g. Similarly, for MCK/LpL0 mice that have no endogenous mouse LpL activity and survive through the expression of human LpL primarily in skeletal muscle, the uptake of ^{3}H label in the heart was lower than in WT or MCK/LpL2 mice. This difference between MCK/LpL0 and WT mice was statistically significant (1.02 ± 0.33% of dose in WT mice versus 0.47 ± 0.21% of dose in MCK/LpL0 mice). Uptake of ^{3}H label by intraperitoneal fat was not different for MCK/LpL0 mice from WT mice nor was uptake of ^{3}H label by subcutaneous fat from MCK/LpL0

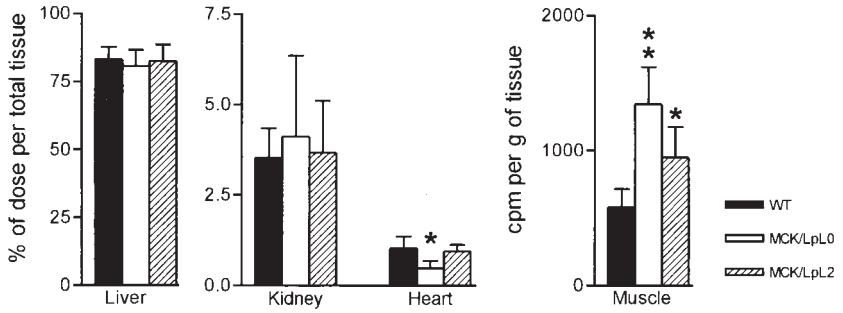

Fig. 2. Tissue sites of uptake of [^{3}H]retinyl ester from rat mesenteric chylomicrons 20 min after injection into fasted WT (■), MCK/LpL0 (□), and MCK/LpL2 (▨) mice. The values are given either as the percentage of ^{3}H cpm present in the dose taken up by the tissue after normalization for recovery of ^{3}H cpm in all tissues studied or as ^{3}H cpm present per g of tissue. Four animals were analyzed for each strain. Values given for kidney represent the ^{3}H cpm taken up by both kidneys. These values are taken from one experiment (the same as for Fig. 1) which was carried out in duplicate and save the same results. Error bars indicate ± 1 standard deviation. *Significantly ($P < 0.05$) different from WT mice. **Significantly ($P < 0.01$) different from WT mice.

mice different than that of WT or MCK/LpL2 mice. Although the amount of ^{3}H label taken up by adipose tissue for MCK/LpL0 mice tended to be lower than those observed for WT and MCKLpL2 mice, this was not significant. Uptake of ^{3}H label by liver and kidney (Fig. 2) and lung and spleen (data not shown) were not significantly different for the three strains.

Effects of LpL expression on steady state tissue retinol and retinyl ester levels

As the greatest differences in uptake of ^{3}H labeled retinoid from chylomicrons was observed for the WT and MCK/LpL0 mice, we asked whether steady state tissue retinoid levels differed between these two strains of mice. **Table 1** shows the total tissue retinol concentrations (expressed as μg retinol equivalents per gram tissue) for 7 tissues. The total retinol contents of liver, lung, perirenal fat, spleen, kidney, skeletal muscle, and heart were not statistically different for the two strains of mice. As some of these tissues in MCK/LpL0 mice take up significantly more postprandial retinoid than do corresponding tissues in WT mice, this would suggest that the retinoid taken up postprandially by these tissues must equilibrate with retinoid pools throughout the body.

Clearance of [^{3}H]retinyl ester in rat chylomicrons by fed and fasted rats

We next investigated the effects of feeding and fasting on the clearance of chylomicrons containing radiolabeled retinyl ester in the rat. It is known that in the rat LpL activity increases in heart and skeletal muscle and decreases in adipose tissue during fasting (26–28). Making use of these differences in LpL activity in the fasted and fed states, we investigated the rate of plasma clearance and tissue sites of uptake of the radiolabeled retinoid in rats subjected to these nutritional states.

Figure 3 and **Fig. 4** show the results of an experiment in which fed and fasted rats were injected with chylomicrons containing [^{3}H]retinyl esters. Four rats were fasted 24 h prior to injection of a bolus dose of the labeled chylomicrons and 4 rats were allowed free access to chow during this period. Each rat received 0.4 mL of the labeled rat chylomicrons containing 12 mg triglyceride and 1.7 μCi (46 nmol) [^{3}H]retinyl ester. The amount of ^{3}H radioactivity remaining in the serum compartment was determined 5, 10, and 20 min after injection (Fig. 3). As seen in **Figure 3**, for each of these 3 times, the amount of ^{3}H label present in the plasma of fasted rats was significantly less than in fed rats. For both fasted and fed rats, less than 10% of the total ^{3}H dose administered remained in the serum after 20 min.

TABLE 1. Steady state tissue retinoid levels for WT and MCK/LpL0 mice

Tissue	WT	(n)	MCK/LpL0	(n)
	μg ROH eq/g		*μg ROH eq/g*	
Liver	560.4 ± 277.7	5	543.6 ± 150.6	6
Lung	308.3 ± 211.0	5	397.8 ± 131.5	6
Perirenal fat	3.6 ± 1.1	5	3.7 ± 1.0	6
Spleen	1.3 ± 0.7	9	0.8 ± 0.5	10
Kidney	0.4 ± 0.2	9	0.3 ± 0.1	10
Skeletal muscle	0.3 ± 0.2	9	0.4 ± 0.3	10
Heart	0.1 ± 0.1	4	0.1 ± 0.1	4

All values are given as the mean ± 1 SD. Statistically significant differences between WT and MCK/LpL0 total retinol levels were not observed.

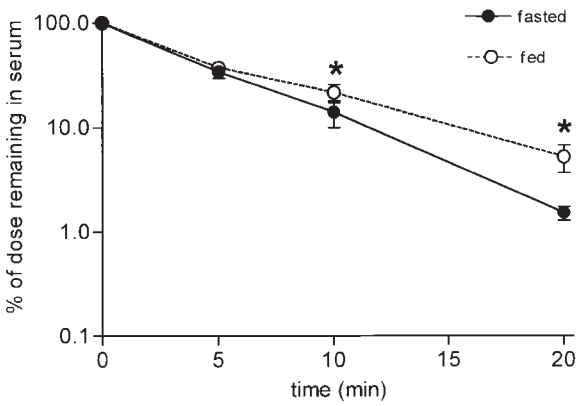

Fig. 3. Plasma decay curve for [^{3}H]retinyl ester-labeled rat mesenteric chylomicrons injected into the circulations of fasted (●) and fed (○) rats. The values represent the percent of the injected ^{3}H dose remaining in the circulation at each time point. The serum volume of rats is taken as 2.75% of total body weight (20). Four animals were analyzed in each group. If an error bar is not visible, it is within the border of the symbol. The dose of chylomicrons consisted of 12 mg TG and 1.7 μCi (46 nmol) [^{3}H]retinyl ester per animal. This experiment is representative of duplicate experiments. *Significantly ($P < 0.05$) different from value in fasted rats.

Figure 4 shows the tissue sites of uptake of the ^{3}H label from chylomicrons from the fed and fasted rats at the time of killing (20 min after injection of the bolus dose) for different tissues. The total ^{3}H cpm recovered for the tissues examined were calculated and these were not significantly different for fasted and fed rats (63 ± 2% and 64 ± 5%, respectively). For both fasted and fed rats, uptake of the ^{3}H label was highest in the liver, averaging 89% of the recovered dose for fasted rats and 85% for fed rats, a difference that is not statistically significant. Uptake of [^{3}H]retinoid by heart was significantly lower in fed rats (0.16 ± 0.08% of the administered dose fed compared to 0.40 ± 0.06% for fasted rats). The epididymal fat pads from fed rats accumulated statistically greater amounts of ^{3}H cpm than the corresponding fat depot from fasted rats. Although skeletal muscle appeared to take up less of the ^{3}H label in the fed state as compared to the fasted state these differences were not statistically significant (122 ± 45 ^{3}H cpm/g for fasted and 67 ± 30 ^{3}H cpm/g for the fed state). No differences in uptake of the ^{3}H label upon fasting or feeding were observed for spleen, lung and kidney (data not shown).

Clearance of chylomicrons containing [^{3}H]retinyl ester in fasted rats and in fasted rats receiving heparin intravenously

In order to investigate further the mechanisms through which feeding status modulates uptake of chylomicron [^{3}H]retinoid, we explored chylomicron uptake by tissues of fasted rats and fasted rats receiving a simultaneous in-

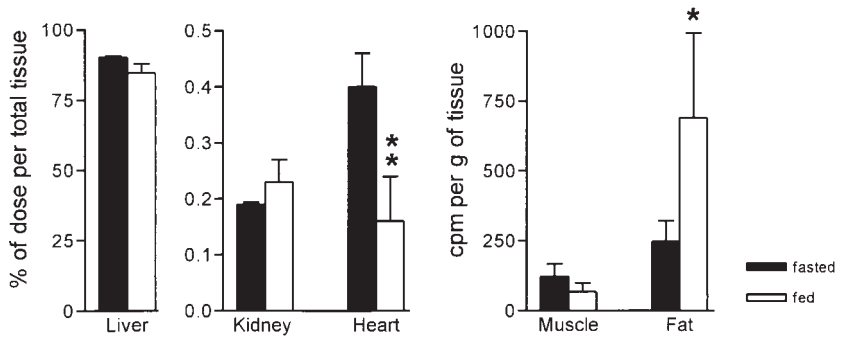

Fig. 4. Tissue sites of uptake of [^{3}H]retinyl ester from rat chylomicrons, 20 min after injection into fasted (■) or fed (□) rats. The values represent either the percent of the ^{3}H cpm present in the dose taken up in each tissue after normalization for the total recovery of ^{3}H label or the total ^{3}H cpm present per g of tissue. Four animals were analyzed in each group. The values presented for kidney were measured for one kidney. The error bar represents 1 standard deviation. If an error bar is not visible, it is within the border of the bar graph. The dose of chylomicrons consisted of 12 mg triglyceride and 1.7 μCi (46 nmol) [^{3}H]retinyl ester per animal. *Significantly ($P < 0.05$) different from the level observed in fasted rats. **Significantly ($P < 0.01$) different from the level observed in fasted rats.

travenous injection of heparin (100 IU/kg). As heparin is known to enhance serum LpL activity by releasing the enzyme from binding sites on the endothelial cell surface (29, 30), we wanted to understand whether LpL catalytic activity is important for facilitating chylomicron retinoid clearance. **Figure 5** shows the serum clearance curves of ^{3}H cpm for both groups of rats. As seen in Fig. 5, for fasted animals, approximately 35% of the injected dose of ^{3}H dose remained in the serum at 5 min after injection whereas for fasted rats receiving the heparin injection the amount of ^{3}H label in the serum had only decreased to 4%. Similar differences in the clearance of chylomicron core triglycerides have been reported by others for rats given an injection of heparin simultaneously with a chylomicron dose (30).

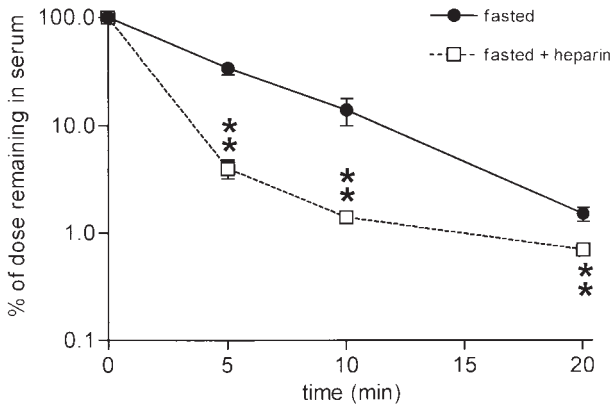

Fig. 5. Plasma decay curve for [^{3}H]retinyl ester-labeled rat mesenteric chylomicrons into the circulations of fasted rats with (□) or without (●) simultaneous injection of 100 IU/kg heparin. The values represent the percent of the ^{3}H cpm in the dose remaining in the circulation at each time. Four animals were analyzed in each group. The error bar represents 1 standard deviation. If an error bar is not visible, it is within the border of the symbol. The dose of chylomicrons injected into each animal consisted of 12 mg triglyceride and 1.7 μCi (46 nmol) [^{3}H]retinyl ester. **Significantly ($P < 0.01$) different from that of fasted rats.

Figure 6 shows the tissue distribution of accumulated ^{3}H cpm 20 min after injection of the chylomicron dose. The recovery of radiolabeled retinoid was not significantly different for the tissues from heparin-treated or untreated rats and averaged 61 ± 4% and 63 ± 2%, respectively. Livers of fasting heparin injected rats accumulated significantly more label, 94 ± 0.5% of the recovered dose of ^{3}H label, as compared to fasting sham-injected rats (which accumulated 89 ± 2.0% of the dose in liver). This difference although relatively small was statistically significant. The accumulation of ^{3}H label by heart muscle in fasted rats injected with heparin was significantly lower (0.15 ± 0.02%) than in sham-injected fasted animals (0.40 ± 0.06%) and similar to that which we observed for fed rats (0.16 ± 0.08%) (see Fig. 4). The same pattern of heparin injection seen for heart was also observed for skeletal muscle where the differences between heparin and sham-injected rats reached statistical significance. Uptake of [^{3}H]retinyl ester by epididymal fat, spleen, lung, and kidney from fasted rats was unaffected by heparin injection (data not shown).

Hydrolysis of chylomicron [^{3}H]retinyl ester in serum of rats

As LpL is able to hydrolyze chylomicron retinyl ester in vitro (14), the possibility of in situ hydrolysis of chylomicron [^{3}H]retinyl ester by LpL was investigated. Twenty minutes after a bolus intravenous injection of chylomicrons containing [^{3}H] retinyl ester into fed and fasted rats with or without a simultaneous injection of heparin, the level of [^{3}H]retinol in serum was determined by HPLC. For both fed and fasted rats not provided a simultaneous injection with heparin, no detectable [^{3}H]retinol and only [^{3}H]retinyl ester was observed in the serum. Interestingly, [^{3}H]retinol was detected in serum of fasted rats receiving heparin. At this time, approximately 0.7% of the total ^{3}H dose still remained in the serum and [^{3}H]retinol accounted for 22.5 ± 6.5% (n = 4) of the [^{3}H]retinoid still present in the circulations of these animals (the remainder of the ^{3}H-cpm was present as retinyl ester). This

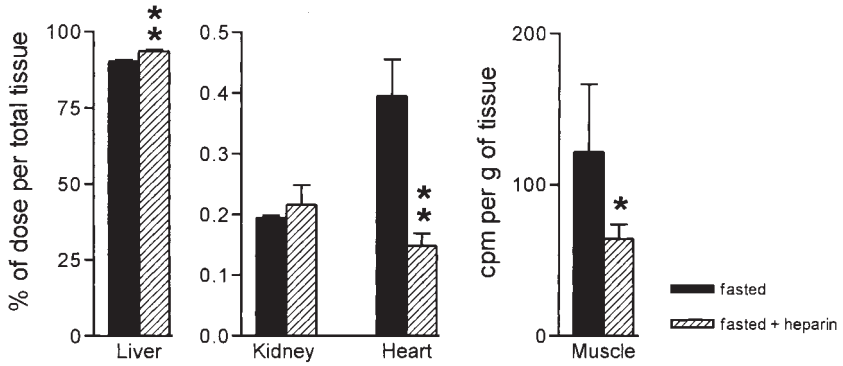

Fig. 6. Tissue sites of uptake of [^{3}H]retinyl ester from rat mesenteric chylomicrons 20 min after injection into fasted rats without heparin injection (□) or fasted rats simultaneously injected with 100 IU/kg heparin (▨). The values represent either the percent of the ^{3}H cpm injected in the dose taken up by a each tissue after normalization for the total recovery of ^{3}H cpm or the ^{3}H cpm present per g of tissue. Four animals were analyzed in each group. The values presented for kidney were measured for one kidney. The error bar represents 1 standard deviation. If an error bar is not visible, it is within the border of the bar graph. The dose of chylomicrons injected into each animal consisted of 12 mg triglyceride and 1.7 μCi (46 nmol) [^{3}H]retinyl ester. *Significantly ($P < 0.05$) different from that of fasted rats. **Significantly ($P < 0.01$) different from that of fasted rats.

observation is consistent with the hypothesis that chylomicron remnant retinyl ester can undergo hydrolysis in the circulation. However, it cannot be taken as conclusive in vivo evidence for LpL-catalyzed hydrolysis of chylomicron retinyl esters as it is possible that some [^{3}H]retinol was resecreted from the liver after chylomicron remnant uptake.

Clearance of [^{3}H]retinyl palmitate and its nonhydrolyzable retinyl [^{14}C]hexadecyl ether analog from Intralipid emulsion in fasted rats

In order better to understand whether retinyl ester hydrolysis is a prerequisite for LpL-mediated clearance of retinoid from chylomicrons and their remnants, we investigated the uptake of emulsion particles that had been doubly labeled with [^{3}H]retinyl palmitate and its nonhydrolyzable ether analog retinyl [^{14}C]hexadecyl ether. As the labeling of rat chylomicrons simultaneously with retinyl ester and retinyl ether is technically complex and can only be accomplished with considerable in vitro manipulation, we choose to label Intralipid emulsion with these retinoids. This decision was based on understanding that fat emulsions like the commercially available Intralipid emulsion are metabolized through processes involving LpL and in a manner that is similar to those of endogenous chylomicrons (20, 31). For these studies, 4 fasted rats were injected with a bolus dose consisting of Intralipid emulsion containing 4 mg triglyceride, 0.8 μCi (0.015 nmol) [^{3}H]retinyl palmitate, and 0.15 μCi (0.57 nmol) retinyl [^{14}C]hexadecyl ether. Timed blood sampling indicated that the rate of clearance of [^{3}H]retinyl palmitate from the circulation was not significantly different from that of the non-hydrolyzable retinyl [^{14}C]hexadecyl ether.

The emulsion particles containing ^{3}H-labeled retinyl ester and ^{14}C-labeled retinyl ether injected into the experimental animals had a ^{3}H cpm/^{14}C cpm ratio of 1.19. We reproducibly found much more of the ^{3}H label than ^{14}C label was taken up by skeletal muscle. For skeletal muscle extracts, the ^{3}H cpm/^{14}C cpm ratio exceeded 19.10 for each animal studied. For adipose tissue, the mean observed ^{3}H cpm/^{14}C cpm ratio was 3.10 and for heart this mean ratio was 1.52. In keeping with this relatively greater uptake of ^{3}H cpm by these extrahepatic tissues, the ^{3}H cpm/^{14}C cpm ratio determined for liver was 0.96. We believe that these data support the notion that hydrolysis of dietary retinyl ester is important for retinoid uptake by skeletal muscle, adipose tissue, and heart. However, we also would point out that, aside from these tissues, we found that other tissues took up nearly equal amounts of the two radiolabels, suggesting that retinyl ester hydrolysis may not always be necessary for uptake by these other tissues.

For liver, lung, and spleen, the degree of intracellular hydrolysis of the [^{3}H]retinyl ester was investigated using a normal phase HPLC procedure able to separate retinol, retinyl esters, and the retinyl hexadecyl ether. This experiment was carried out in part to provide a basis for comparison of our data, obtained with Intralipid emulsions, with that of Goodman, Huang, and Shiratori (2) in studies of chylomicron retinoid clearance in rats. Specifically, we wanted to confirm that after uptake by cells the emulsion retinyl ester is processed in a manner that is similar to what has been observed for retinyl ester arriving in chylomicrons (2). Twenty min after injection of the dual-labeled emulsion, 23% of the [^{3}H]retinyl ester taken up by the liver was hydrolyzed to [^{3}H]retinol. [^{3}H]Retinol accounted for 21% of the total [^{3}H]retinoid present in lung and approximately 42% of the total [^{3}H]retinoid present in spleen. The values for liver and lung agree well with those of Goodman and colleagues (2) who found that 0.28 h after a bolus injection approximately 20% of the chylomicron retinoid taken up by liver and lung is present as retinol. Although Goodman and colleagues (2) did not explore retinoid uptake in spleen, we have found spleen to be an interesting tissue with regards to chylomicron retinoid uptake as the spleen is even more active in hydrolyz-

ing incoming dietary retinyl esters than the liver. As expected, all of the ^{14}C cpm present in these tissues was present as the intact retinyl [^{14}C]hexadecyl ether.

DISCUSSION

The work of Goodman, Stein, and colleagues (2–4) and others (1, 6, 7) in the mid-1960s established that dietary retinoid is taken into the body along with other dietary lipids as a component of chylomicrons and that about three quarters of the chylomicron retinoid is ultimately taken up by the liver. This early work also demonstrated that about one quarter of the chylomicron retinoid is reproducibly taken up by extrahepatic tissues, mostly by skeletal muscle, heart, adipose tissue, and kidney (2). There have been many advances in our understanding of chylomicron formation, metabolism, and clearance from the circulation in the years since these seminal early studies. Extensive information is now available regarding the roles of apolipoproteins (A-I, B-48, C-I, C-II, C-III, and E), lipases (hepatic lipase and LpL) and cell surface receptors (LDL receptor, LDL receptor-related protein and the lipolysis stimulated receptor) in facilitating chylomicron metabolism or clearance from the circulation (see refs. 5 and 6 for recent reviews). However, relatively little of this later work has focused specifically on dietary retinoid uptake and/or delivery to tissues. For instance, we presently have only a very limited understanding of the mechanisms or processes through which extrahepatic tissues take up dietary retinoid or of how these respond to changes in physiologic status. Our studies have focused on this area and were designed to provide new insight into the uptake of postprandial retinoid by extrahepatic tissues and into the role played by LpL in facilitating uptake. Overall, our data are consistent with the conclusion that uptake of dietary retinoid by some but not all extraheptic tissues is markedly influenced by tissue levels of LpL.

We have previously reported that LpL is able to hydrolyze chylomicron retinyl ester after most of the triglyceride has first been hydrolyzed and that this activity of LpL enhances uptake of chylomicron retinoid by cultures of BFC-1β adipocytes (14). This led us to suggest that LpL might play an important role in vivo in clearance of dietary retinoid by extrahepatic tissues. This suggestion is supported by our present data obtained from studies of induced mutant mice that express LpL in different anatomic locations and at different levels and from studies of rats manipulated nutritionally or through heparin injection to have different tissue patterns and/or levels of LpL expression. The data supporting this conclusion are most compelling for skeletal muscle. Mice overexpressing human LpL in skeletal muscle take up approximately 2-fold more chylomicron retinoid than do WT mice and manipulations that increase or decrease LpL expression in skeletal muscle of rats correspondingly increase or decrease the amount of chylomicron retinoid taken up by the tissue. In addition, studies in the rat of the clearance of emulsion particles doubly labeled with retinyl palmitate and its non-hydrolyzable retinyl ether analog suggest that hydrolysis of the retinyl ester may be a prerequisite for skeletal muscle uptake as relatively little of the non-hydrolyzable ether analog could be detected in this tissue. Thus, LpL activity in skeletal muscle would seem to be a key determinant of the amount of dietary retinoid taken up by this tissue.

Interestingly, for other tissues that express LpL, this conclusion is not always valid. The kidney, for instance, expresses LpL but for none of the different experimental conditions that we investigated did we observe any difference in the amount of retinoid taken up by kidney. We take this to indicate that the actions of LpL in facilitating uptake of chylomicron retinoid are mechanistically complex and are tissue and cell type specific (see below for more details). A similar conclusion was reached for studies of chylomicron uptake by rabbit liver and bone marrow (32).

Both adipose tissue and heart express LpL. For these tissues, the data are generally in keeping with our finding for skeletal muscle that the level of LpL expression in the tissue directly influences retinoid uptake by the tissue. In a statistically significant manner, epididymal fat accumulated more retinoid in the fed state, when LpL activity levels are high in this tissue, than in the fasted state and this effect was abolished upon heparin injection to displace LpL anchored to the vessel walls of the adipose capillary beds. Adipose tissue from mice that do not express LpL in this tissue (the MCK/LpL0 mice) took up on average less than half of chylomicron retinoid than observed for wild type mice. However, because of the relatively large error associated with these measures, this difference could not be concluded as statistically significant. For heart, retinoid uptake was significantly lower when LpL expression levels were low and high when expression levels were high, both in genetically manipulated mice and in nutritionally manipulated rats. Thus, the pattern of retinoid uptake from chylomicron and emulsion particles by heart and adipose tissue is consistent between experiments and in general agreement with the conclusion made for skeletal muscle that the level of LpL expression in the tissue directly influences retinoid uptake by the tissue.

There are several mechanisms that could account for the uptake of the retinyl ester by LpL expressing tissues. First, the retinyl ester could be hydrolyzed, as we observed in in vitro studies (14), and the free retinol could diffuse into the tissues. Second, retinyl ester could dissociate from the chylomicron and then be internalized as a component of the surface lipid that is shed from the chylomicron during lipolysis. Third, the entire chylomicron or chylomicron remnant might associate with LpL and then be internalized either via classical receptors or along with recycling of cell surface proteoglycans. Or fourth, it is conceivable that the core retinyl ester can be exchanged for another non-polar lipid at or near the cell surface. Of these mechanisms, the first two require the enzymatic actions of LpL to hydrolyze triglyceride or retinyl ester within the chylomicron. The last two mechanisms involve the non-enzymatic 'bridging' functions of LpL. The data

comparing the uptake of the non-hydrolyzable retinyl ether and the retinyl ester by skeletal muscle suggest that retinoid uptake involves, at least to some degree, the enzymatic actions of the enzyme. As this experiment was performed using smaller emulsion size particles rather than rat chylomicrons, it may be that enzymatic-mediated uptake is only seen with small particles that are relatively triglyceride depleted. The final proof that non-enzymatic LpL actions operate in vivo will require studies in transgenic mice containing enzymatically inactive LpL.

As we have pointed out above in the Results section, some extrahepatic tissues that do not express LpL (specifically in LpL-deficient mice overexpressing human LpL in skeletal muscle) were also found to take up postprandial retinoid. Thus, some extrahepatic tissues are able to take up chylomicron retinoid through LpL-independent mechanisms. Several mechanisms may account for this uptake. First, it is possible that some retinyl ester could dissociate from the chylomicrons (or chylomicron remnants) and then be internalized as a component of the surface lipid that is shed from the particle as it traverses the vascular compartment. Alternatively, it is possible that the entire chylomicron or chylomicron remnant can be internalized via classical receptors or along with normal recycling of cell surface proteoglycans. Most likely, the postprandial retinoid taken up by extrahepatic tissues in an LpL-independent manner arises from both of these processes.

The early studies of Goodman and colleagues (2) in the rat indicated that between 3 to 7.3% of the [^{3}H]retinoid present in a bolus dose of chylomicrons is taken up by skeletal muscle. Our data for skeletal muscle retinoid uptake for both mice and rats fall within this range. Yet, our data on steady state hepatic and skeletal muscle total retinol concentrations (see Table 1) indicate that skeletal muscle contains only approximately 1% of the total retinol present in the mouse liver. As steady state total retinol levels for these tissues are not commensurate with the relative percentage of postprandial retinoid accumulated, this would suggest that dietary retinoid which is taken up by skeletal muscle must be mobilized from the tissue for transport to the liver and possibly to other tissues for storage. As uptake of dietary retinoid would be continuously occurring in skeletal muscle, the process of retinol mobilization would similarly need to be continuously ongoing. Based on the literature, this indeed seems likely. Green, Uhl, and Green (33), Green, Green, and Lewis (34), and Lewis et al. (35), using tracer kinetic approaches, have reported that retinol is recycled 6 times between the periphery and the liver before it is irreversibly lost from the body of a rat. Soprano and Goodman (36) have reported that skeletal muscle in the rat expresses plasma RBP, albeit at levels which are less than 1% those of the liver. Thus, when our data on chylomicron retinoid uptake by extrahepatic tissues are considered in light of information already available in the literature, it would not seem unreasonable that dietary retinoid is continuously being taken up by extrahepatic tissues like the skeletal muscle and continuously being recycled back to the liver for storage and eventual resecretion into the circulation.

Surprisingly, although overexpression of LpL in muscle increased retinoid uptake from postprandial lipoproteins, this did not affect tissue steady state retinoid content. Therefore, for these tissues, retinoid stores did not correlate with uptake of chylomicron retinyl ester. This suggests that tissue content of retinoids is dependent on other pathways of retinoid uptake or on the capacity of the tissues to store this fat-soluble vitamin. Retinol that circulates as the retinol-RBP complex is the major plasma delivery pathway for retinoids and is the major source of retinoid delivery to many tissues (19). Intracellular retinol is either associated with cellular retinol-binding proteins (7) or with fat droplets (7). Thus, the capacity of cells for retinoid storage, rather than retinol uptake, might be the primary determinants of cellular and tissue retinoid content. We would speculate that cellular and tissue capacities for retinoid storage are dependent on levels of cellular retinol-binding protein, type I and/or lecithin:retinol acyltransferase activity within the cell or tissue.

From the perspective of retinoid biology, possibly the most important question that emerges from our observations concerns the physiological significance of delivery of dietary retinoid to extrahepatic tissues. It has long been clear from the literature that extrahepatic tissues are able to take up dietary retinoid. Our studies confirm and extend this early observation by establishing that the amount of dietary retinoid taken up by some extrahepatic tissues directly depends on the level of LpL expression by the tissue. In addition, our data establish that uptake of chylomicron retinoid is responsive to normal changes in physiologic status such as fasting and feeding. At a minimum, the uptake of chylomicron retinoid by extrahepatic tissues must be viewed as an alternative pathway through which tissues can acquire retinoid needed to maintain normal gene expression, in essence, an alternative to delivery as retinol bound to RBP or as retinoic acid bound to albumin (7, 8). Although this delivery pathway is certainly a redundant one for delivery of retinoid to tissues, it is nevertheless a pathway through which tissues can acquire retinoid. It is possible that chylomicron retinoid may serve a very specific functional role in some tissues under some physiological or pathological contexts. It is clear that retinoic acid plays an important role in the development of muscle tissue (37), in maintaining adipose tissue activities (38), and in development of the heart (39), and it is probable that retinoic acid is needed to maintain the normal health of each of these tissues. It seems likely that the retinyl ester present in chylomicrons can serve as a direct precursor for the synthesis of this retinoic acid in these tissues. At present, our data do not provide insight into whether retinoid delivered via chylomicrons serves a specific functional role within tissues like skeletal muscle, adipose tissue, and heart or whether this delivery is more simply a redundant mechanism for keeping tissues supplied with needed retinoid. Insight into this possibility is needed if we are to understand fully the importance of chylomicron delivery of postprandial retinoid to extrahepatic tissues.

The authors wish to thank Dr. Maysoon Al-Haideri for her expert advice regarding the labeling and preparation of In-

tralipid emulsions. We also wish to acknowledge the assistance of Dr. Jan L. Breslow of Rockefeller University for making this work possible. This work was supported by grants DK52444 (WSB), HL45095 (IJG), HL54591(to Dr. Jan L. Breslow), and DK44498 (EHH) from the National Institutes of Health.

Manuscript received 14 July 1998 and in revised form 4 November 1998.

REFERENCES

1. Goodman, D. S., and W. S. Blaner. 1984. Biosynthesis, absorption, and hepatic metabolism of retinol. *In* The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, New York. 1–39.
2. Goodman, D. S., H. S. Huang, and T. Shiratori. 1965. Tissue distribution and metabolism of newly absorbed vitamin A in the rat. *J. Lipid Res.* **6:** 390–396.
3. Huang, H. S., and D. S. Goodman. 1965. Vitamin A and carotenoids I. Intestinal absorption and metabolism of ^{14}C-labeled vitamin A alcohol and β-carotene in the rat. *J. Biol. Chem.* **240:** 2839–2844.
4. Stein, O., Y. Stein, D. S. Goodman, and N. H. Fidge. 1969. The metabolism of chylomicron cholesteryl ester in rat liver. A combined radioautographic-electron microscopic and biochemical study. *J. Cell Biol.* **43:** 410–431.
5. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
6. Cooper, A. D. 1997. Hepatic uptake of chylomicron remnants. *J. Lipid Res.* **38:** 2173–2192.
7. Blaner, W. S., and J. A. Olson. 1994. Retinol and retinoic acid metabolism. *In* The Retinoids, Biology, Chemistry and Medicine. 2nd ed. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Raven Press, New York, NY. 282–301.
8. Soprano, D. R., and W. S. Blaner. 1994. Plasma retinol-binding protein. *In* The Retinoids, Biology, Chemistry and Medicine. 2nd ed. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Raven Press, New York, NY. 257–281.
9. Camps, L., M. Reina, M. Lobera, S. Vilaro, and T. Olivecrona. 1990. Lipoprotein lipase: cellular origin and functional distribution. *Am. J. Physiol.* **258:** C673–C681.
10. Khoo, J. C., E. Mahoney, and J. L. Witztum. 1981. Secretion of lipoprotein-lipase by macrophages in culture. *J. Biol. Chem.* **256:** 7105–7108.
11. Chait, A., P. H. Iverius, and J. D. Brunzell. 1982. Lipoprotein lipase secretion by human monocyte-derived macrophages. *J. Clin. Invest.* **69:** 490–493.
12. Lookene A., R. Savonen, and G. Olivecrona. 1997. Interaction of lipoproteins with heparan sulfate proteoglycans and with lipoprotein lipase. Studies by surface Raman resonance technique. *Biochemistry.* **36:** 5267–5275.
13. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
14. Blaner, W. S., J. C. Obunike, S. B. Kurlandsky, M. Al-Haideri, R. Piantedosi, R. J. Deckelbaum, and I. J. Goldberg. 1994. Lipoprotein lipase hydrolysis of retinyl ester: possible implications for retinoid uptake by cells. *J. Biol. Chem.* **269:** 16559–16565.
15. Hultin, M., R. Savonen, and T. Olivecrona. 1996. Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling. *J. Lipid Res.* **37:** 1022–1036.
16. Weinstock, P. H., C. L. Bisgaier, I. K. Aalto-Set, H. Radner, R. Ramakrishnan, S. Levak-Frank, A. D. Essenburg, R. Zechner, and J. L. Breslow. 1995. Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. *J. Clin. Invest.* **96:** 2555–2568.
17. Bridges, C. D., and R. A. Alvarez, 1982. Measurement of the vitamin A cycle. *Methods Enzymol.* **81:** 463–485.
18. Goodman, D. S., O. Stein, G. Halperin, and Y. Stein. 1983. The divergent metabolic fate of ether analogs of cholesteryl and retinyl esters after injection in lymph chylomicrons into rats. *Biochim. Biophys. Acta.* **750:** 223–230.
19. Kurlandsky, S. B., M. V. Gamble, R. Ramakrishnan, and W. S. Blaner. 1995. Plasma delivery of retinoic acid to tissues in the rat. *J. Biol. Chem.* **270:** 17850–17857.
20. Hultin, M., A. Mollertz, M. A. Zundel, G. Olivecrona, T. T. Hansen, R. J. Deckelbaum, Y. A. Carpentier, and T. Olivecrona. 1994. Metabolism of emulsions containing medium- and long-chain triglycerides or interesterified triglycerides. *J. Lipid Res.* **35:** 1850–1860.
21. Kaluzny, M. A., L. A. Duncan, M. V. Merritt, and D. E. Epps. 1985. Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J. Lipid Res.* **26:** 135–140.
22. Lenich, C. M., and A. C. Ross. 1987. Chylomicron remnant-vitamin A metabolism by the human hepatoma cell line HepG2. *J. Lipid Res.* **28:** 183–194.
23. Mills, J. L., J. Tuomilehto, K. F. Yu, N. Colman, W. S. Blaner, P. Koskela, M. S. Bundle, M. Forman, L. Toivanen, and G. G. Rhodes. 1992. Maternal vitamin levels during pregnancies producing infants with neural tube defects. *J. Pediatr.* **120:** 863–871.
24. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
25. Levak-Frank, S., P. H. Weinstock, T. Hayek, R. Verdery, W. Hofmann, R. Ramakrishnan, W. Sattler, J. L. Breslow, and R. Zechner. 1997. Induced mutant mice expressing lipoprotein lipase exclusively in muscle have subnormal triglycerides yet reduced high density lipoprotein cholesterol levels in plasma. *J. Biol. Chem.* **272:** 17182–17190.
26. Doolittle, M. H., O. Ben-Zeev, J. Elovson, D. Martin, and T. G. Kirchgessner. 1990. The response of lipoprotein lipase to feeding and fasting. *J. Biol. Chem.* **265:** 4570–4577.
27. Liu, G., and T. Olivecrona. 1992. Synthesis and transport of lipoprotein lipase in perfused guinea pig hearts. *Am. J. Physiol.* **263:** H438–H446.
28. Linder, C., S. S. Chernick, T. R. Fleck, and R. O. Scrow. 1976. Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats. *Am. J. Physiol.* **231:** 860–864.
29. Chevreuil, O., M. Hultin, P. Ostergaard, and T. Olivecrona. 1993. Biphasic effects of low-molecular-weight and conventional heparins on chylomicron clearance in rats. *Arterioscler. Thromb.* **13:** 1397–1403.
30. Tornvall, P., G. Olivecrona, F. Karpe, and A. Hamsten. 1995. Lipoprotein lipase mass and activity in plasma and their increase after heparin are separate parameters with different relations to plasma lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1086–1093.
31. Oliveira, H. C., M. H. Hirata, T. G. Redgrave, and R. C. Maranhao. 1988. Competition between chylomicrons and their remnants for plasma removal: a study with artificial emulsion models of chylomicrons. *Biochim. Biophys. Acta.* **958:** 211–217.
32. Hussain, M. M., I. J. Goldberg, K. H. Weisgraber, R. W. Mahley, and T. L. Innerarity. 1997. Uptake of chylomicrons by the liver, but not by the bone marrow, is modulated by lipoprotein lipase activity. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1407–1413.
33. Green, M. H., L. Uhl, and J. B. Green. 1985. A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores. *J. Lipid Res.* **26:** 806–818.
34. Green, M. H., J. B. Green, and K. C. Lewis. 1987. Variation in retinol utilization rate with vitamin A status in the rat. *J. Nutr.* **117:** 694–703.
35. Lewis, K. C., M. H. Green, J. B. Green, and L. A. Zech. 1990. Retinol metabolism in rats with low vitamin A status: a compartmental model. *J. Lipid Res.* **31:** 1535–1548.
36. Soprano, D. R., K. J. Soprano, and D. S. Goodman. 1986. Retinol-binding protein messenger RNA levels in the liver and in extrahepatic tissues of the rat. *J. Lipid Res.* **27:** 166–171.
37. Alric, S., A. Froeschle, C. Piquemal, G. Carnac, and A. Bonnieu. 1998. Functional specificity of the two retinoic acid receptor RAR and RXR families in myogenesis. *Oncogene.* **16:** 273–282.
38. Schoonjans, K., J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. 1996. *EMBO J.* **15:** 5336–5348.
39. Zile, M. H. 1998. Vitamin A and embryonic development: an overview. *J. Nutr.* **128:** 455S–458S.