Evidence for distinct metabolic utilization of stearic acid in comparison with palmitic and oleic acids in rats

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Stearic acid, unlike other saturated fatty acids, is possibly hypolipidemic. The rate of plasma clearance and hepatic metabolism of stearic acid, as incorporated into chylomicron lipids, was compared with those of palmitic and oleic acids. Chylomicrons were specifically enriched and labeled in vivo with ^{14}C-stearic acid (SA), palmitic (PA), or oleic acid (OA). Following intravenous injection of rats with labeled chylomicrons, the rates of plasma clearance and hepatic incorporation of the label in triglyceride (TG), phospholipid (PL), and other lipids were compared at 5-, 15-, and 30-min intervals. Stearic acid was cleared at the slowest rate ($t_{1/2}$ = 19.7 min) compared with PA ($t_{1/2}$ = 6.2 min) and OA ($t_{1/2}$ = 8.1 min). At 30 min, only 68% of the SA dose was removed from the plasma, whereas most of PA (96%) and OA (92%) was cleared. SA and OA were removed by the liver at significantly slower rates compared with PA. At the peak (15 min) of plasma clearance, 17–18% of SA and OA were found in the liver, whereas 37% of PA was taken up by the liver. In the liver, 31.8% of the labeled SA and smaller fractions of PA (15.1%) and OA (6.8%) were present in PL. Conversely, less of the hepatic SA (31.4%) was found in TG, compared with PA (49.0%) and OA (60.4%) in the liver. No significant difference was noted in the relative distributions of ^{14}C in cholesterol and other lipids. Conversion of SA to OA in the liver and intestine was minimal. The data provide new evidence that SA is released slowly from chylomicrons and preferentially utilized for PL synthesis in the liver. The results also suggest that OA is less contributory to hepatic lipogenesis because it is removed by the liver at a much slower rate and better utilized by the extrahepatic tissue than SA and PA. Among the three fatty acids compared, PA is most rapidly taken up and incorporated into TG in the liver. This may stimulate the hepatic synthesis of very low density lipoproteins, (VLDL), contributing to hyperlipidemia. (J. Nutr. Biochem. **4**:594–601, 1993.)

Keywords: chylomicrons; fatty acid; oleic acid; palmitic acid; stearic acid

Introduction

Studies with various animal models have demonstrated that stearic acid (SA), unlike other long chain saturated fatty acids, lowers plasma cholesterol and is less atherogenic, as reviewed by Kritchevsky.[1] More recent human studies[2–4] also have provided evidence that diets high in SA significantly reduce the plasma levels of total cholesterol and low density lipoprotein (LDL) cholesterol, compared with those high in palmitic acid (PA).

The mechanism whereby SA lowers plasma cholesterol is not well understood. It has been demonstrated that fats high in SA are incompletely digested and less efficiently absorbed.[5,6] However, several studies involving human subjects [2–4] indicate that the relative absorption of SA is similar to that of PA or oleic acid (OA). Therefore, the inefficient intestinal absorption of SA alone may not explain its cholesterol-lowering effect. Alternatively, diets high in SA have been shown to lower the intestinal absorption of cholesterol in rats.[5] Also, it has been suggested that SA, once absorbed, may be readily converted by desaturation to OA, which is known for its hypocholesterolemic effect.[2–4]

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Thus far, however, little information exists concerning the metabolic fate of SA immediately after it enters the circulation postabsorptively. The present study was designed to examine possible distinct behavior of SA during the early phase of its metabolism, as it is transported by chylomicrons into the blood. To achieve this aim, chylomicrons were specifically enriched and labeled in vivo with ^{14}C-SA, PA, or OA. The rates of plasma clearance and hepatic utilization of the three fatty acids were compared.

Methods and Materials

Animals and diet

Fifty-one mature male albino rats with an average weight of 250 g (Harlan Sprague Dawley, Inc. Indianapolis, IN USA) were housed individually in stainless-steel cages and subjected to a cycle of a 1500–0300 light and 0300–1500 dark periods. Temperature and humidity were controlled at 23–25° C and 55–75%, respectively. The animals were cared for in an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care facility at Kansas State University and fed a nutritionally adequate diet formulated according to the recommendations by the American Institute of Nutrition,[7,8] except for its fat content. The diet, as shown in *Table 1*, contained 5% beef tallow in addition to the 5% corn oil, as recommended by the AIN. This modification was made to better simulate the amount and type of dietary fat present in a typical American diet. All animals were fed the diet ad libitum for 4 weeks and allowed free access to deionized water throughout the experiment.

Cannulation of mesenteric lymph duct

At 4 wk, six rats weighing approximately 340 ± 21 g were used as donors from which labeled chylomicrons were obtained. The mesenteric lymph duct was cannulated by a modification of the procedure described in our previous studies.[9,10] Briefly, after fasting for 24 hr, rats were anesthetized with halothane. An abdominal incision was made along the midline by using a cauterizer. The major intestinal lymph duct was cannulated with vinyl tubing (SV. 31 tubing; ID, 0.50 mm; OD, 0.80 mm, Dural Plastics, Auburn, Australia). An indwelling infusion catheter (Silastic medical grade tubing; ID, 1.0 mm; OD, 2.1 mm, Dow Corning, Midland, MI USA) was placed via the gastric fundus into the upper duodenum and secured by a purse-string suture (4-0 Silk, Ethicon, Somerville, NJ USA). After the abdominal incision was closed, the rats were placed in stainless-steel restraining cages in a heated chamber (30° C) for postoperative recovery for 24 hours. During this period, the rats were infused via the duodenal catheter with a maintenance solution consisting of 5% glucose, 0.87% NaCl, and 0.03% KCl at the rate of 2.5 mL/hr by using an infusion pump (Auto-syringe Inc. Model 5B, Hookset, NH USA).

Table 1 Composition of experimental diet

Ingredient	g/Kg
Egg whites, spray dried	200
Corn starch	100
Dextrose	502.996
Cellulose	50
Corn oil	50
Beef Tallow	50
Mineral mix*	35
Vitamin mix*	10
Biotin	0.004
Choline bitartrate	2

*According to the recommendations of the American Institute of Nutrition.[7,8]

Preparation and labeling of chylomicrons

Following postoperative recovery of rats, six lymph-fistula rats were divided into three groups. To produce chylomicrons enriched and labeled with a specific fatty acid, two donor rats were infused intraduodenally via the catheter with a lipid emulsion (infusate) enriched with SA, PA, or OA. The infusate enriched with a specific fatty acid was obtained by mixing 18–20 mL of Intralipid (10% soybean oil, 1.2% egg yolk phospholipid, and 2.25% glycerin, USP; KabiVitrum, Alameda, CA USA) containing 10 mg α-tocopherol and an emulsion of SA, PA, or OA prepared by sonication in 0.75 g Na-taurocholate in 40–42 mL phosphate-buffered saline (6.75 mM Na_2HPO_4, 16.5 mM, NaH_2PO_4, 115 mM NaCl, 5 mM KCl, and 2.5 g glucose, pH 6.4). The total concentration of the fatty acid (SA, PA, or OA) in the mixture was maintained at 400 mg per 60 mL. After adding 15–25 μCi of the specific ^{14}C-fatty acid, the mixture was sonicated (W-375, Heat Systems-Ultrasonics, Long Island, NY USA). The radiochemical purities of the ^{14}C-labeled fatty acids (NEN, Du Pont, Wilmington, DE USA; specific activities, 56–58 mCi/mmol) were 98.9–99.0%, as determined by thin layer chromatography (TLC) on silica gel G with hexane: diethylether: acetic acid (70:30:1).

After a steady flow of lymph (2.5–3.0 mL/hr) was established by infusing the maintenance solution during the postoperative recovery period, the donor rats were infused via the duodenal catheter with the lipid infusate at 2.5 mL/hr. Lymph was collected for about 20 hours at 30° C in a sterilized 50-mL plastic tube. The lymph was defibrinated by passing through glass wool. The filtrate was overlayered with 150 mM NaCl (pH 7.4) in polyallomer tubes and centrifuged at 1.3 × 10^6 *g*/min at 28° C using a Beckman 50.3 Ti rotor in a Beckman L5-75B ultracentrifuge (Spinco Division, Palo Alto, CA USA). The packed top fraction of chylomicron was separated by draining the tube after puncturing the bottom and dispersed in 0.87% NaCl, pH 7.3, by passage through a 23-gauge needle at room temperature. Approximately 89% of the labeled SA was found in triglyceride (TG) and 9% in phospholipid (PL), whereas 95% of the ^{14}C-PA and OA was present in TG and 1–2% in PL, with the remainder in the fractions of diglyceride plus cholesterol and cholesterol ester (*Table 2*). Distributions

Table 2 Distribution (%) of ^{14}C radioactivity among different lipid classes in chylomicrons produced by infusing the duodenal infusates

	TG*	PL	DG + CH	CE
OA†	95.1	1.2	3.4	0.3
PA	94.7	1.7	3.3	0.3
SA	88.7	8.7	2.3	0.3

*TG, triglyceride; PL, phospholipid; DG + CH, diglyceride and cholesterol; CE, cholesterol ester.
†Chylomicrons labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid); and SA (^{14}C-stearic acid).

of fatty acids in the infusates and chylomicrons were determined by gas chromatography using a Carbowax column (15 cm × 0.53 mm ID). The fatty acid composition of chylomicrons (*Table 3*) closely reflected that of the infusate.

Determination of the rate of plasma clearance

Forty-five recipient rats were fasted for 10 hr and divided into three groups of 15 rats each. Each of the three groups was injected with a dose of chylomicrons labeled with ^{14}C-SA, PA, or OA. The labeled chylomicrons were preheated at 60° C for 2 min under N_2 and cooled to 37° C prior to injection. Previously,[11] it was found that reheating to 58° C was necessary to restore the spherical shape of the lymph lipoproteins rich in saturated fat exposed to temperatures at 23–26° C. Rats were closely matched between groups with regard to their body weight. The average body weights for SA, PA, and OA groups were 321 ± 13, 318 ± 11, and 318 ± 13 g, respectively. The rates of plasma clearance of the labeled chylomicrons were determined by injecting recipient rats via a jugular vein with a dose of chylomicrons. Each dose consisted of 80 mg of labeled chylomicrons in 200 μL of 150 mM NaCl (pH 7.4). Five rats from each group were killed under ether anesthesia at 5, 15, and 30 min after dosing. Blood samples were withdrawn, and the livers removed. Blood samples (2 mL) were collected via the orbital sinus[12] and plasma was separated by centrifugation at 1000*g* for 60 min. Aliquots (100–200 μL) of plasma were mixed with scintillation fluid (Scintiverse, Fisher Scientific, Chicago, IL USA) and counted to determine the plasma ^{14}C radioactivity (Beckman LS 8000, Beckman Instruments, Fullerton CA USA). The total volume of plasma was calculated as 3.35% of the body weight, as determined by a radioisotopic dilution method in our previous study.[13] Percentage of the dose cleared at each time interval was computed by (^{14}C dpm at t_i/^{14}C dpm at t_0) × 100, where t_i is a given time interval and t_0 is time of dosing. The rate of clearance for each fatty acid was expressed by half time ($t_{1/2}$), which is the amount of time taken for ^{14}C radioactivity to decrease to one-half of the total ^{14}C radioactivity injected. The $t_{1/2}$ was determined by the equation for the rate of an exponential change, $t_{1/2} = -0.693/k$, where k is the slope of the time-course clearance curve of ^{14}C radioactivity.

Distribution of ^{14}C radioactivity in different lipid classes in the liver

The liver removed after exsanguination was blotted with absorbent paper, weighed, and frozen at −70° C until analysis. The whole liver was finely minced with a razor blade. A 2-g sample was taken and used for extraction of lipids by the method of Folch et al.[14] The total ^{14}C radioactivity of the liver was determined by counting an aliquot of extracted lipids and expressed as percent of the dose injected. Distribution of ^{14}C radioactivity in different lipid classes was determined by separating the liver lipids by thin layer chromatography (TLC) on silica gel G (20 × 20 cm, 250 microns, Analtech, Newark, DE USA) with n-hexane-diethylether-glacial acetic acid (70:30:2, vol/vol/vol). The solvent system gave an excellent separation of cholesterol ester, TG, free (unesterified) fatty acid, monoglyceride, and phospholipid, except for cholesterol and diglyceride, which comigrated and were collected as one spot. Separated lipids were visualized with iodine vapor. The lipid spots on TLC plates were scraped into counting vials and eluted with 1.0 mL of 100% ethanol for 10 min. The radioactivity of each lipid class was counted, as above. The distribution (percent) of ^{14}C radioactivity among liver lipids was calculated, and the fatty acid composition of liver lipids was also determined by gas chromatography.

Distribution of ^{14}C radioactivities between saturated and unsaturated fatty acids

To examine whether ^{14}C-saturated fatty acids infused are desaturated during chylomicron synthesis in the intestinal mucosa and after their uptake into the liver, chylomicron and liver lipid extracts were further saponified and methylated.[15] Methylesters of the fatty acids were separated into saturated and unsaturated fractions by TLC on silver-nitrate–impregnated Silica Gel G plates (10% $AgNO_3$, 250 microns, 20 × 20 cm, Analtech, Newark, DE USA). The plates were developed in 100% chloroform, and the lipid spots visualized by spraying water. The separated lipids were scraped, eluted with 100% ethanol, and counted for ^{14}C radioactivity.

Statistics

Analysis of variance (ANOVA) with the least significant difference (LSD) test of the SAS statistical package (SAS, Inc. Cary, NC USA) was used to determine differences ($P < 0.05$) between group means. All data were expressed as mean ± SD.

Results

Comparison of the rates of plasma clearance

Figure 1 shows the time-course ^{14}C-clearance curves for the labeled fatty acids. ^{14}C-SA was cleared from the plasma at a markedly slower rate, compared with ^{14}C-PA and ^{14}C-OA during the 30-min period. The rates ($t_{1/2}$) of clearance during the 30-min period for ^{14}C-SA, ^{14}C-PA, and ^{14}C-OA were 19.7 ± 8.2, 6.2 ± 0.3, and 8.1 ± 1.8 min, respectively. PA was cleared most rap-

Table 3 Fatty acid compositions (%) of the duodenal infusates and ^{14}C-labeled chylomicrons produced by infusing the duodenal infusates

		16:0	18:0	18:1	18:2	18:3	20:4
Infusate	OA*	12.1	4.8	23.3	52.9	6.9	—
	PA	19.3	4.7	21.5	48.1	6.2	—
	SA	11.4	14.0	21.1	47.3	6.1	—
Chylomicron	OA†	12.2	6.4	23.2	50.0	5.8	2.3
	PA	14.7	3.0	22.6	51.5	6.3	2.0
	SA	15.3	14.2	20.5	42.8	4.5	2.7

*Duodenal infusates labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid); and SA (^{14}C-stearic acid).
†Chylomicrons labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid); and SA (^{14}C-stearic acid).

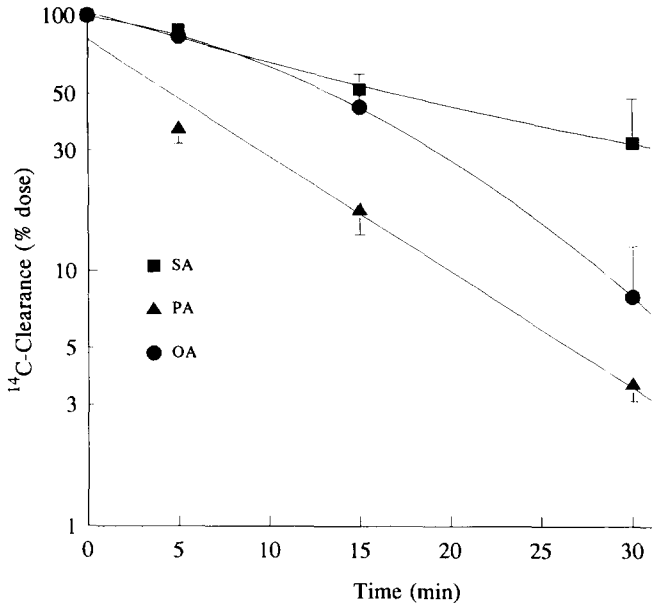

Figure 1 Time-course clearance of ^{14}C-labeled stearic, palmitic, and oleic acids, incorporated into chylomicron lipids. The rates ($t_{1/2}$: half-life) of clearance for ^{14}C-stearic, oleic, and palmitic acid were 19.7 ± 8.2, 8.1 ± 1.8, and 6.2 ± 0.3 min, respectively. Stearic acid was cleared at a significantly slower rate ($P < 0.05$), compared with palmitic and oleic acids.

idly. ^{14}C-SA and ^{14}C-OA were removed at a similar rate up to 15 min. At 30 min, however, ^{14}C-SA was removed at a significantly slower rate than OA. At 5 min, 63.5% of ^{14}C-PA was cleared from the plasma, whereas only 12.5% of ^{14}C-SA and 17.0% of ^{14}C-OA were removed. At 15 min, 83% of ^{14}C-PA, 48% of ^{14}C-SA, and 56% of ^{14}C-OA were removed. At 30 min, most (94–96%) of ^{14}C-PA and ^{14}C-OA were cleared, while 68% of ^{14}C-SA was removed from the plasma.

Comparison of the hepatic uptake and utilization

Figure 2 compares the ^{14}C radioactivities appearing in the liver at 5, 15, and 30 min. The hepatic ^{14}C radioactivities, as expressed in percent dose, generally reflected the rates of clearance of the labeled fatty acids. ^{14}C-PA was most rapidly removed by the liver, whereas ^{14}C-OA was taken up at the slowest rate. At 15 min, 37.3% of ^{14}C-PA was found in the liver, while 17–18% of ^{14}C-SA and OA were recovered in the liver. At 30 min, 34.1% of ^{14}C-PA, 28.6% of ^{14}C-SA, and 18.4% of ^{14}C-OA were recovered in the liver. The hepatic recoveries of ^{14}C radioactivities did not account for possible losses of ^{14}C due to β-oxidation within the liver. The ^{14}C uptake by the peripheral (extrahepatic) tissue was estimated by the initial ^{14}C dose minus the ^{14}C radioactivities recovered in the liver and remaining in the plasma. The ^{14}C uptake by the peripheral tissue at each time interval is shown in *Table 4*. At 5 min postdosing, 44.5% of ^{14}C-PA, 8.2% of ^{14}C-OA, and a trace of ^{14}C-SA were attributed to the peripheral uptake. The peripheral removal of ^{14}C-OA was markedly accelerated at 15 min and thereafter. At 30 min, 76.0% of ^{14}C-OA, 62.4% of ^{14}C-PA, and 39.3% of ^{14}C-SA were removed by the peripheral tissue.

The percentages of the ^{14}C dose appearing in monoglyceride, diglyceride plus cholesterol, and cholesterol ester in the liver are shown in *Table 5*. Regardless of the fatty acids, less than 3% of the ^{14}C dose was found in the combined fractions of monoglyceride, diglyceride, and free and esterified cholesterol during the 30-min period. Much of the injected ^{14}C-dose was associated with free (unesterified) fatty acid (FFA), PL, and TG. The time-course changes in the ^{14}C-radioactivities appearing in FFA, TG, and PL are presented graphically by *Figure 3*. In general, the amounts of ^{14}C ap-

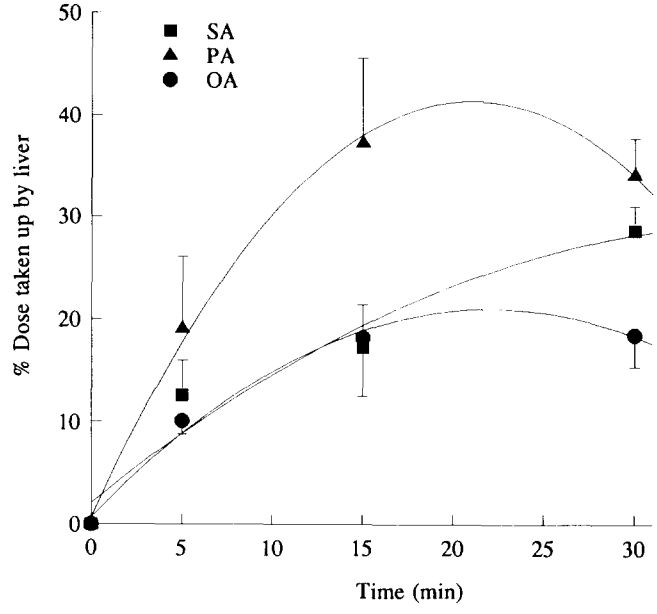

Figure 2 Hepatic uptake (% dose) of ^{14}C-stearic (SA) , oleic (OA), and palmitic (PA) acids at different time intervals following intravenous injection of the chylomicrons labeled with the ^{14}C-fatty acids.

Table 4 Percentages of the ^{14}C doses removed by the liver and the extrahepatic tissue at different time intervals following injection of doses of chylomicrons labeled with ^{14}C-oleic, palmitic, or stearic acid*

	Time intervals after injection of ^{14}C-fatty acids		
	5 min	15 min	30 min
Hepatic			
OA†	10.2 ± 1.1^{a*}	18.2 ± 5.1^{a}	18.4 ± 2.7^{a}
PA	19.0 ± 6.3^{b}	37.3 ± 7.4^{b}	34.1 ± 3.2^{b}
SA	12.5 ± 3.0^{ab}	17.2 ± 3.7^{a}	28.6 ± 2.3^{c}
Extrahepatic‡			
OA	8.2 ± 5.3^{a}	37.5 ± 13.5^{ab}	75.9 ± 4.0^{a}
PA	44.5 ± 5.2^{b}	45.3 ± 7.0^{a}	62.4 ± 3.6^{a}
SA	0.0^{c}	30.9 ± 6.1^{b}	39.3 ± 16.5^{b}

*Mean ± SD of five rats. Values not sharing common superscripts within the same column are significantly different ($P < 0.05$).
†Chylomicrons labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid), and SA (^{14}C-stearic acid).
‡Extrahepatic uptake calculated as 100 − (% total dose in liver + % total dose remaining in plasma).

Table 5 Percentages of the ^{14}C doses appearing in monoglyceride, diglyceride plus cholesterol, and cholesterol ester fractions in the liver at different time intervals after injection of doses of chylomicrons labeled with ^{14}C-oleic, palmitic, or stearic acid*

	MG	DG + CH	CE
5 min			
OA†	0.1 ± 0.0[a]	0.6 ± 0.1[a]	0.2 ± 0.0[a]
PA	0.3 ± 0.2[a]	1.3 ± 0.5[b]	0.1 ± 0.0[a]
SA	0.3 ± 0.2[a]	1.1 ± 0.3[b]	0.3 ± 0.2[a]
15 min			
OA	0.3 ± 0.1[a]	1.1 ± 0.4[a]	0.4 ± 0.2[a]
PA	0.6 ± 0.3[b]	2.6 ± 0.3[b]	0.3 ± 0.1[a]
SA	0.5 ± 0.1[b]	1.3 ± 0.3[a]	0.1 ± 0.2[a]
30 min			
OA	0.4 ± 0.1[a]	1.1 ± 0.2[a]	0.7 ± 0.3[a]
PA	1.0 ± 0.4[b]	2.0 ± 0.1[b]	0.5 ± 0.2[a]
SA	1.0 ± 0.4[b]	1.6 ± 0.4[a]	0.6 ± 0.4[a]

*Values not sharing common superscripts within the same lipid class are significantly different ($P < 0.05$).
†Chylomicrons labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid); and SA (^{14}C-stearic acid).
MG, monoglyceride; DG + CH, diglyceride and cholesterol; and CE, cholesterol ester.

pearing in FFA in the liver (upper panel, *Figure 3*) closely reflected the rates of liver uptake of the labeled fatty acids. The radioactivity of unesterified ^{14}C-PA accumulated most rapidly, with a plateau at 15 min followed by a precipitous decline at 30 min. A similar pattern of change was noted in the radioactivity of unesterified ^{14}C-OA. The radioactivity of unesterified ^{14}C-SA rose linearly with time, indicating a continuous influx of the fatty acid into the liver from the plasma for up to 30 min.

The extents to which the ^{14}C-fatty acids were incorporated into TG and PL were markedly different. In contrast to ^{14}C-SA, more of ^{14}C-PA and ^{14}C-OA were incorporated into TG (middle panel, *Figure 3*). At 30 min, 16.6% of the total dose of ^{14}C-PA and 11.2% of ^{14}C-OA were found in TG, whereas 9.0% of the dose of ^{14}C-SA was incorporated into TG. Among the three fatty acids, ^{14}C-PA was most rapidly utilized for TG synthesis, whereas ^{14}C-SA was most slowly assembled into TG in the liver. The incorporation of ^{14}C-SA and ^{14}C-PA into PL was linearly increased with time, whereas ^{14}C-OA was minimally incorporated into PL (lower panel, *Figure 3*). Compared with ^{14}C-PA and ^{14}C-OA, significantly higher percentages of ^{14}C-SA were incorporated into PL at all intervals. At 30 min, 9.1% of the dose of ^{14}C-SA was found in PL, while 5.1% of ^{14}C-PA and only 1.2% of ^{14}C-OA were incorporated into PL.

Distribution of ^{14}C radioactivity between saturated and unsaturated fatty acids

In both chylomicron and liver lipids, 100% of ^{14}C-OA appeared in the unsaturated fatty acid fraction (*Table 6*). Greater than 95% of ^{14}C-PA and ^{14}C-SA were dis-

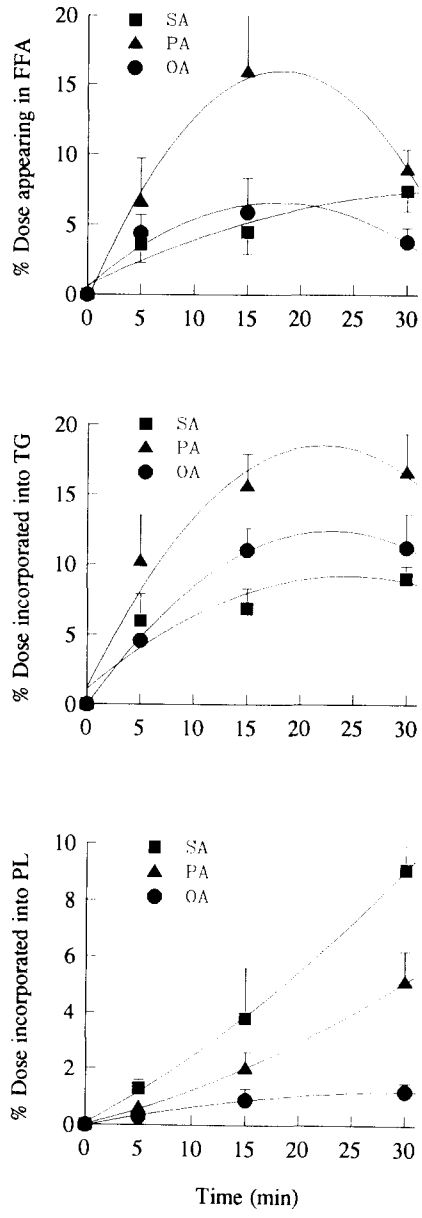

Figure 3 (Upper panel) Time-course appearance (% dose) of ^{14}C-stearic (SA), oleic (OA), and palmitic acid (PA) in the liver as free fatty acids at different time intervals following injection of chylomicrons labeled with the ^{14}C-fatty acids. At 5 min, no difference was noted between the groups in the ^{14}C radioactivity appearing in the liver. At 15 min, the radioactivity of ^{14}C-PA was significantly higher relative to that of ^{14}C-OA or ^{14}C-SA. At 30 min, the radioactivities of ^{14}C-PA and ^{14}C-SA were higher than that of ^{14}C-OA, with no difference between ^{14}C-PA and ^{14}C-SA. (Middle panel) Incorporation (% dose) of ^{14}C-stearic (SA), oleic (OA), and palmitic (PA) acids into triglyceride at different time intervals following injection of chylomicrons labeled with the fatty acids. A significantly greater incorporation of ^{14}C-PA into TG was observed at all time intervals, compared with ^{14}C-SA and ^{14}C-OA. At 15 and 30 min, the incorporation of ^{14}C-OA into TG was significantly greater than that of ^{14}C-SA. ^{14}C-SA was least utilized for TG synthesis among the fatty acids compared. (Lower panel) Incorporation (% dose) of ^{14}C-stearic (SA), oleic (OA), and palmitic (PA) acids into phospholipid at different time intervals following injection of chylomicrons labeled with the ^{14}C-fatty acids. A significantly greater incorporation of ^{14}C-SA into PL was evident, compared with ^{14}C-OA and ^{14}C-PA. Significantly smaller fractions (% dose) of ^{14}C-OA were incorporated into PL at 15 and 30 min, compared with ^{14}C-PA and ^{14}C-SA.

Table 6 Distribution (%) of the ^{14}C radioactivities of injected chylomicron and liver lipids between saturated and unsaturated fatty acids

	Chylomicron		Liver removed at 5 min		Liver removed at 15 min		Liver removed at 30 min	
	SAT	UNS	SAT	UNS	SAT	UNS	SAT	UNS
OA*	0	100	0	100	0	100	0	100
PA	99.2	0.8	98.0 ± 1.2	2.0 ± 1.2	97.9 ± 2.4	2.1 ± 2.4	99.2 ± 1.7	0.8 ± 1.7
SA	98.7	1.3	99.4 ± 1.2	0.6 ± 1.2	95.5 ± 6.6	4.5 ± 6.6	94.8 ± 6.0	5.2 ± 6.0

*Chylomicrons labeled with OA (^{14}C-oleic acid), PA (^{14}C-palmitic acid); and SA (^{14}C-stearic acid).
SAT, saturated fatty acid fraction; UNS, unsaturated fatty acid fraction.

tributed in the saturated fatty acid fraction. The data indicated that neither ^{14}C-SA nor ^{14}C-PA was desaturated or converted to ^{14}C-OA to significant extents in the intestine or the liver.

Discussion

Using chylomicrons labeled in vivo with specific ^{14}C-fatty acids, the present study has demonstrated that during the early postabsorptive phase SA, PA, and OA are metabolized in distinctly different patterns in rats. In this study, we have presented the following new findings: (1) stearic acid is removed from the plasma at a slower rate, relative to PA and OA; (2) stearic acid is preferentially utilized for PL synthesis in the liver; (3) stearic acid is not converted to OA to a significant extent during chylomicron synthesis in the intestinal mucosa and after its uptake by the liver; (4) palmitic acid is most rapidly removed by the liver and favorably incorporated into TG; (5) Oleic acid is better utilized by the peripheral tissue. In the liver, it is largely incorporated into TG and minimally into PL.

Stearic acid, as incorporated into chylomicron lipids, is cleared from plasma at a markedly slower rate ($t_{1/2}$ = 19.7 min), compared with PA ($t_{1/2}$ = 6.2 min) and OA ($t_{1/2}$ = 8.1 min). The reason for this slower rate of clearance is not readily apparent. Upon their entry into the plasma, chylomicrons are subjected to lipolysis by lipoprotein lipase on the endothelial surface of the peripheral tissue, with resultant formation of chylomicron remnants. The remnant particles, enriched in apolipoprotein (apo) E, are then rapidly removed by the liver via a high affinity apo E receptor.[16,17] The rate of plasma clearance of chylomicron fatty acid, therefore, is determined by the rates of both peripheral lipolysis and hepatic uptake. The present data indicate that the slower clearance of SA is mainly due to a slower rate of peripheral lipolysis and uptake. During the initial 5 min, little or no ^{14}C-SA in chylomicron lipids was removed peripherally, whereas nearly 45% of ^{14}C-PA was taken up. Although the peripheral removal of ^{14}C-SA was accelerated with time, it remained significantly lower than that of ^{14}C-PA. At 30 min, only 39% of the dose of ^{14}C-SA was attributable to the extrahepatic uptake in contrast to 62% of ^{14}C-PA. This may be related to the difference in chain length of the fatty acids.[18,19] Unlike these saturated fatty acids, ^{14}C-OA was most rapidly removed by the peripheral tissue, but most slowly taken up by the liver, suggesting that the degree of saturation of the fatty acyl moieties is another important determinant of the rate of plasma clearance. A previous study[19] under in vitro conditions showed that saturated fatty acyl chains were hydrolyzed by lipoprotein lipase at rates 5–10 times slower than unsaturated fatty acyl esters. Mortimer et al.[20] showed that a chylomicron-like emulsion containing 1,3-dioleoyl-2-stearoylglycerol (OSO) was cleared more slowly from the plasma than an emulsion containing triolein (OOO). Similarly, lymph chylomicrons obtained from donor rats fed OSO were cleared at a slower rate than those produced from OOO.[21] It has been postulated that 2-stearoylglycerol, a product of OSO hydrolysis by lipoprotein lipase, creates a more rigid and tightly packed surface on a remnant particle. Such surface characteristics of the particle may reduce its affinity for apo C-II or lipoprotein lipase, thereby retarding further lipolysis and hepatic uptake.[20,21]

Another important factor contributing to the slower clearance of ^{14}C-SA may be its favored incorporation into PL during chylomicron formation in the intestinal mucosa. Our data indicated that a significantly greater proportion of ^{14}C-SA is incorporated into chylomicron PL when the fatty acid was infused intraduodenally. Approximately 9% of the label (^{14}C) was incorporated into PL and 89% into TG. In contrast, only 1–2% of ^{14}C-PA and ^{14}C-OA was found in PL and 94–95% in TG (*Table 2*). It is well established that during peripheral lipolysis the majority of chylomicron PL are readily transferred to plasma high density lipoproteins (HDL).[22,23] The half-life ($t_{1/2}$) of HDL phospholipid is estimated to be 40 min.[24] Thus, it is probable that most of the ^{14}C-SA incorporated into chylomicron PL was acquired by HDL and the label (^{14}C) transferred to the HDL surface was removed at a much slower rate. On the other hand, ^{14}C-PA and ^{14}C-OA were mostly incorporated into chylomicron TG and more rapidly removed by the liver via the high-affinity chylomicron remnant (or apoE) receptor. This finding strongly suggests that the manner in which a dietary fatty acid is packaged into specific chylomicron lipids at the intestinal level may be an important determinant of the rate of its clearance from the plasma and its subsequent delivery into the liver and other tissues.

After it was taken up by the liver, ^{14}C-SA was most rapidly incorporated into PL. When expressed in per-

cent of the ^{14}C dose, the total incorporations of labeled SA, PA, and OA into PL were 9.1, 5.1, and 1.2%, respectively. Among the three fatty acids, SA was least utilized for TG synthesis (*Figure 3*). Increased incorporation of ^{14}C-SA into PL was observed despite its slower rate of uptake by the liver. Although ^{14}C-OA was taken up at a similar rate during the initial 15 min, its appearance in PL was minimal. The appearance of ^{14}C-SA in PL increased markedly with time. At 30 min, 32% of the hepatic ^{14}C-SA was incorporated into PL, whereas only 15% of ^{14}C-PA and 7% of ^{14}C-OA appeared in this lipid. The observed differences in their incorporation into PL were not related to differences in dilution of the fatty acid labels in the liver. The percent distributions of total SA, PA, and OA in the liver were 15.0 ± 2.7, 17.5 ± 1.9, and 14.2 ± 1.1%, respectively. Previously, Leyton et al.[25] and Elovson[26] also demonstrated a rapid and preferential incorporation of stearic acid into liver PL after oral and intraportal administration of the fatty acid. Because of its favored utilization for PL synthesis in the liver, SA probably is less stimulatory to hepatic synthesis of triglyceride (lipogenesis) and more favorably incorporated into cellular membranes and lipoprotein coats.

In contrast to ^{14}C-SA and OA, ^{14}C-PA is more rapidly removed by the liver and preferentially utilized for TG synthesis in the liver. During a 30-min period, 17% of the total ^{14}C-PA was incorporated into TG, in comparison to 9% of ^{14}C-SA and 11% of ^{14}C-OA. Reflecting its rapid clearance from the plasma, the radioactivity of unesterified ^{14}C-PA rose sharply in the liver during the first 15 min and then precipitously declined, largely because of its rapid incorporation into TG at 30 min. During this period, the loss of ^{14}C radioactivity due to β-oxidation was expected to be minimal. In another study,[25] only 0.3–0.5% of ^{14}C-SA and ^{14}C-PA were found to be lost by oxidation, as measured by $^{14}CO_2$ released in intact rats at 60 min after dose administration. Nearly half (49%) of the hepatic ^{14}C-PA was utilized for TG, whereas a significantly smaller fraction (15%) was used for PL synthesis. The findings indicate that PA is more contributory to hepatic lipogenesis. This may be associated with the rapid rate of its hepatic uptake and hence increased availability of the substrate for TG synthesis. Such metabolic behavior of PA is conducive to an increased synthesis and release of VLDL from the liver, which, in turn, may contribute to hyperlipidemia. A previously study[27] showed that the dietary addition of PA accelerates the secretion of TG and cholesterol from the liver via very low density lipoprotein (VLDL).

Compared with ^{14}C-SA and PA, ^{14}C-OA was taken up by the extrahepatic (peripheral) tissue or oxidized at an increasingly faster rate with time. Consequently, only 18% of the dose of ^{14}C-OA was found in the liver at 30 min, compared with 34% of ^{14}C-PA and 29% of ^{14}C-SA. Relative to ^{14}C-PA, ^{14}C-OA is more slowly incorporated into liver TG, largely because of its slower uptake by the liver and better utilization by the peripheral tissue or oxidation within the liver. Its incorporation into PL was minimal and did not increase appreciably with time. Leyton et al.[25] also reported that ^{14}C-OA is oxidized at a faster rate than ^{14}C-SA and ^{14}C-PA, as measured in vivo by the recovery of expired $^{14}CO_2$. These findings indicate that OA is more rapidly oxidized and, therefore, less stimulatory to hepatic lipogenesis.

Previously, it has been postulated that dietary SA, once absorbed, may be readily converted OA, thus exerting a hypolipidemic effect.[2–4] The present data show that SA is not converted to OA to a significant extent in either intestine or liver. The results clearly indicate that SA does not behave metabolically like OA. They are distinctly different in regard to the rate of clearance, the site of metabolism, and the extent to which they are utilized for the synthesis of PL or TG.

In summary, using chylomicrons labeled in vivo with specific fatty acids, the present study has provided new evidence for distinct utilization and metabolic behavior of SA in comparison to PA and OA. Stearic acid, as incorporated into chylomicron lipids, is removed from the plasma at a markedly slower rate. Once taken up by the liver, it is preferentially utilized for PL synthesis. Its hypolipidemic effect does not appear to be related to its conversion to oleic acid, but to its favored utilization for PL synthesis. In contrast, PA is most rapidly taken up and utilized for TG synthesis in the liver. This finding suggests that PA is more stimulatory to the hepatic synthesis of VLDL, contributing to hyperlipidemia. The present data also show that OA is more rapidly oxidized by the extrahepatic tissue and/or in the liver. Oleic acid is less contributory to hepatic lipogenesis than PA, and is least utilized for PL synthesis among the three fatty acids compared.

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