Simultaneous measurements of chylomicron lipolysis and remnant removal using a doubly labeled artificial lipid emulsion: studies in normolipidemic and hyperlipidemic subjects

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Abstract An artificial chylomicron-like lipid emulsion doubly labeled with tri[(N)^{3}H]oleoylglycerol ([^{3}H]TO) and cholesteryl [1-^{14}C]oleate ([^{14}C]CO) was infused intravenously into human subjects with the purpose of simultaneously measuring the plasma disappearance rates (residence time, RT) of [^{14}C]CO, which represents solely the splanchnic organ uptake of the remnant chylomicron core, and of [^{3}H]TO, which combines the remnant disappearance with the shedding off of chylomicron triglycerides by the action of lipoprotein lipase. Thus, the fraction of the particle triglyceride content that is removed before the remnant is taken up is expressed as a delipidation index ($DI = 1 - \frac{RT \text{ of } [^3H]TO}{RT \text{ of } [^{14}C]CO}$). The present procedure has an advantage over the use of chylomicrons labeled with retinyl ester or radioactive triglycerides alone that represent, respectively, the chylomicron remnant or the whole particle metabolism only. When normal subjects as well as primary hyperlipidemic subjects were studied, the plasma triglyceride concentration was directly related to [^{14}C]CO RT and [^{3}H]TO RT, but inversely related to the delipidation index. There may be different patterns of relations between these parameters of chylomicron metabolism in primary and in secondary hyperlipidemias, as well as under the action of drugs that influence the metabolism of lipoproteins.—**Nakandakare, E. R., S. A. Lottenberg, H. C. F. Oliveira, M. C. Bertolami, K. S. Vasconcelos, G. Sperotto, and E. C. R. Quintão.** Simultaneous measurements of chylomicron lipolysis and remnant removal using a doubly labeled artificial lipid emulsion: studies in normolipidemic and hyperlipidemic subjects. *J. Lipid Res.* 1994. **35:** 143–152.

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In the blood stream lipoprotein lipase removes fatty acids from triglyceride-rich lipoproteins, generating remnant particles that are taken up by the splanchnic organs. Accumulation of chylomicrons in blood is ascribed to defects in the delipidation process, in the uptake of the remnant particles, or to variable degrees of combinations of both disturbances.

The metabolism of natural chylomicrons has been investigated by labeling chylomicrons with retinyl ester after an oral load of retinol, and the time course of the plasma chylomicron retinyl ester tolerance curve was obtained (1–10). Nonetheless, objections have been raised against the validity of the retinyl ester tolerance curve because, at later times after retinol intake, its ester shifts to plasma lipoproteins at densities heavier than those of the chylomicrons and their remnants (9). Also, autologous retinyl ester-rich chylomicrons were reinfused into the blood stream and the disappearance rate of the retinyl ester-containing particles was measured with time (11–13). Although the retinyl ester of the chylomicrons infused intravenously does not appreciably shift to other plasma lipoprotein fractions (7, 8), the emulsion infused should be regarded as a heterogenous mixture that includes partially metabolized particles, typical chylomicron remnants, and VLDL-like particles produced by the intestine (10–12). An additional inconvenience is that the removal of triglyceride-rich remnants is dependent on the dose infused (12). Finally, the intravenous retinyl ester-containing chylomicron test does not clearly indicate which of the metabolic parameters of chylomicron metabolism may be affected in the hyperlipidemias. In other words, retinyl ester better defines the metabolism of the chylomicron remnants than the whole chylomicron metabolism process which is preceded by delipidation elicited by lipoprotein lipase.

Other techniques for studying chylomicron metabolism include the steady state duodenal infusion of fat (14–17),

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the pulse infusion of large doses of fat emulsions (Intralipid®) (17, 18), or of autologous chylomicrons obtained from the thoracic duct (19), or containing radioactive triglycerides produced after the oral feeding of labeled palmitic acid (20, 21). Although the Intralipid® and the steady duodenal fat infusion methods showed comparable results (17), all of these procedures determine the rate of chylomicron triglyceride removal without distinguishing the process of particle delipidation from that of particle remnant uptake by the splanchnic organs.

Miller and Small (22) have developed artificial emulsions that simulate the size, density, and composition of chylomicrons without apolipoproteins. In experiments conducted on rats, it was shown in vitro that artificial chylomicrons acquire all plasma apolipoproteins that are normal constituents of the chylomicron surface, except for apoB (23). Several studies on rats have validated the use of intravenously infused radioactively labeled artificial chylomicron-like emulsions in the metabolism of natural chylomicrons (24–29). In the present report the alterations of the chylomicron metabolic parameters were measured in primary forms of hyperlipidemia in humans using intravenously pulse-infused artificial chylomicrons containing [^{3}H]TO and [^{14}C]CO. This method permits the simultaneous measurements of two related, although not necessarily interdependent, processes in the metabolism of blood chylomicrons, namely the rates of delipidation and of particle uptake by splanchnic organs. In other words, if the intact particles were removed from plasma without a previous delipidation step, the [^{3}H]triolein/cholesteryl [^{14}C]oleate ratio in plasma would become equal to one in time. Any degree of particle delipidation leads to a plasma [^{3}H]triolein decay curve that is faster than that of cholesteryl [^{14}C]oleate. Because the latter remains in the particle core not appreciably exchanging with other plasma lipoproteins within the time span of the experiment, the cholesteryl [^{14}C]oleate disappearance rate represents the splanchnic uptake of the partially delipidated chylomicron remnant that contains residual [^{3}H]triolein. Therefore, the extent of delipidation is evaluated by the plasma [^{3}H]triolein/cholesteryl [^{14}C]oleate ratio. The rates of delipidation and of particle removal could be affected to different extents according to the type of hyperlipidemia and of hypolipidemic drug used. For instance, although heparin administration markedly accelerates chylomicron lipolysis, it may not necessarily enhance the remnant uptake rate in certain secondary hyperlipidemias where particle recognition by the splanchnic organ receptors is poor. This apparent discrepancy has been shown in studies on normal humans (13) and in experimental rat hyperlipidemias (25, 26).

MATERIAL AND METHODS

Artificial chylomicron preparation

Cholesterol, cholesteryl oleate, and triolein were obtained from Nu-Chek Prep (Elysian, MN) and lecithin was obtained from Lipid Products (Surrey, UK). All were more than 99% pure as shown by TLC. Lipid mixtures (2% cholesterol, 6% cholesteryl oleate, 23% lecithin, and 69% triolein, by weight) were prepared in scintillation vials together with tri[9,10(N)^{3}H]oleoylglycerol and cholesteryl [1-^{14}C]oleate (Amersham International, UK). This mixture was sonicated together with a sodium chloride solution of d 1.101 g/ml using a Branson Cell Disruptor (Branson Ultrasonics Corp, Danbury, CT) model B-30, with a 1-cm probe, submitted to 70–80 watts for 30 min under nitrogen flow, and inside a temperature-controlled water bath.

Artificial chylomicrons were obtained after discontinuous gradient ultracentrifugation of the above emulsion as previously described (30). The final artificial chylomicron composition was: 1.8% cholesterol, 8.9% cholesteryl oleate, 9.3% lecithin, and 80% triolein, by weight.

Protocol of human experiments

Thirty nine adult volunteer subjects were enrolled in this investigation after signing an informed written con-

TABLE 1. Clinical data

Patient	Age	Gender M/F	Body Mass Index[a]	Plasma Lipids	
				Chol	TG
	yr			*mg/dl ± SD*	
Controls	43 ± 11	6/3	25.6 ± 3.6	190 ± 41	118 ± 47
Hypercholesterolemia (primary)	53 ± 15	5/7	25.2 ± 3.1	343 ± 120[b]	120 ± 49
Combined hyperlipidemia (moderate)[c]	47 ± 10	6/4	24.9 ± 2.4	268 ± 47[b]	302 ± 81[d]
Combined hyperlipidemia (severe)[c]	47 ± 17	3/5	23.6 ± 4.0	383 ± 161[b]	1980 ± 1708[d]

Statistical comparison of the groups by ANOVA.
[a]Body mass index = weight (kg)/height (m^2) (31).
[b]Significantly different from controls at $P < 0.01$.
[c]Combined hyperlipidemia was defined solely on the basis of the simultaneous presence of hypercholesterolemia and hypertriglyceridemia. Hypertriglyceridemia was artificially divided into moderate (below 500 mg/dl) and severe (above 500 mg/dl) groups.
[d]Significantly different from controls at $P < 0.001$.

sent according to the protocol approved by the Ethics Committee of the Hospital of the University of São Paulo Medical School. Clinical data and blood lipids in the fasting state are presented in **Table 1.** Hypolipidemic drugs had not been administered for at least 3 months prior to the study.

After an overnight 12-h fast, an antecubital vein was punctured and maintained patent by slow saline infusion while the patients remained seated during the test. Pyrogen-free emulsion was filtered through Millipore disks (0.22 μm) and 0.4–0.6 ml was rapidly infused intravenously. Each patient received approximately 14 μCi tri[9,10(N)^{3}H]oleoylglycerol ([^{3}H]TO) and 3 μCi cholesteryl [1-^{14}C]oleate ([^{14}C]CO).

Blood samples (6 ml) were sequentially drawn at 3, 6, 9, 12, 16, 20, and 30 min into 0.1 ml heparin–10% EDTA solution and immediately centrifuged at 4°C. Plasma was stored at −20°C until further analysis. Triglyceride and cholesteryl ester bands were eluted with ethyl ether from silica gel H TLC plates developed with hexane–ethyl ether–acetic acid 70:30:1(vol/vol). ^{3}H and ^{14}C radioactivity was measured in PPO–POPOP-toluene–phosphor scintillation solution in a beta-scintillation counter (LS-6000 TA, Beckman Instruments Inc., Fullerton, CA).

Calculations

Mean plasma residence time (RT) of cholesteryl [1-^{14}C]oleate and of tri[9,10(N)^{3}H]oleoylglycerol was calculated with time as the area under the plasma radioactivity curve represented as a fraction of the initial value at 3 min (32). A delipidation index (DI) was calculated as proposed by Redgrave and Zech (27), on the assumption that the residence time (RT) of the radioactivity in plasma represents two processes: *1)* [^{14}C]CO-RT estimates the particle removal remnant rate because radioactivity remains in the chylomicron particle during its course of lipolysis in plasma; *2)* [^{3}H]TO-RT results from the combined processes of particle remnant removal and the peeling off of fatty acids. Therefore DI is the fraction of triglycerides shed by the particle as fatty acids before chylomicron internalization by the splanchnic organs. It is calculated as:

$$DI = 1 - \frac{[^3\text{H}]\text{triolein RT}}{\text{cholesteryl } [^{14}\text{C}]\text{oleate RT}}.$$

A hypothetical range of metabolic situations might occur in regard to the plasma decay curves of [^{3}H]TO and of [^{14}C]CO associated with variable rates of particle delipidation as shown schematically in **Fig. 1.** Normally, a fraction of the injected particle triglyceride content leaves the particle before it is taken up by the splanchnic organs: this is exemplified by a delipidation index of 0.3 in panel A. High activity of the enzyme lipoprotein lipase should, in principle, enhance the particle delipidation rate leading to a faster remnant production rate, and consequently the remnant could be available for organ uptake sooner than in the first example: a larger DI value could be simultaneous to a slightly faster plasma decay curve of [^{14}C]CO, but most significantly of [^{3}H]TO. An analogous situation occurs when the particle is poorly recognized by receptors in splanchnic organs, consequently residing in plasma for a much longer period of time while being submitted to the action of the delipidation enzymes (panel B): greater delipidation is achieved (DI = 0.9) and yet the primary defect is represented by a slow removal of [^{14}C]CO plasma. Lack of lipoprotein lipase, as it occurs in Type I hyperchylomicronemia, predictably does not result in shedding of particle [^{3}H]triolein (panel C). Thus, chylomicron remnant production is negligible and the intact particle, that would then be poorly recognized by the tissue receptors, can be removed but at a very slow rate: [^{3}H]TO and [^{14}C]CO residence time values in plasma are considerably high and DI exceedingly low (0.01). Finally, very little particle lipolysis (DI = 0.02 in panel D) may occur simply because the particle removal rate is so fast, for instance, due to a much greater organ lipoprotein receptor number or affinity, or blood flow, that the particle exposure to the peripheral capillary endothelium-attached lipoprotein lipase is extremely short lived: [^{3}H]TO and [^{14}C]CO residence time values are short and similar. All these theoretical situations were indeed found experimentally in artificial chylomicron studies in rats (24–26) and in human studies using retinyl ester labeling of chylomicrons (13).

Chemical analyses

Lipids were measured by enzymatic procedures: cholesterol by the Chod-Pap method (Boehringher Mannheim, Merck SA, R. J., Brazil) and triglycerides by the Enz-color method (Biodiagnostica, SP, Brazil). Phospholipids were measured in the chylomicron preparation by the Bartlett method (33).

RESULTS

Validation of the artificial chylomicron infusion model was supported by lipoprotein analysis of two normal subjects showing that less than 2% of cholesteryl [^{14}C]oleate and none of the [^{3}H]triolein was present at plasma densities greater that 1.006 g/ml at 20 and 30 min after chylomicron infusion.

Results from some individual doubly labeled chylomicron-infused patients are presented in **Fig. 2** which illustrates the plasma [^{3}H]TO and [^{14}C]CO radioactivity curves (dpm/ml). Panel A presents a normolipidemic subject whose delipidation index was 0.36. In panel B, a hypothyroidism case is shown with a greater DI value, seemingly secondary to a slower chylomicron remnant

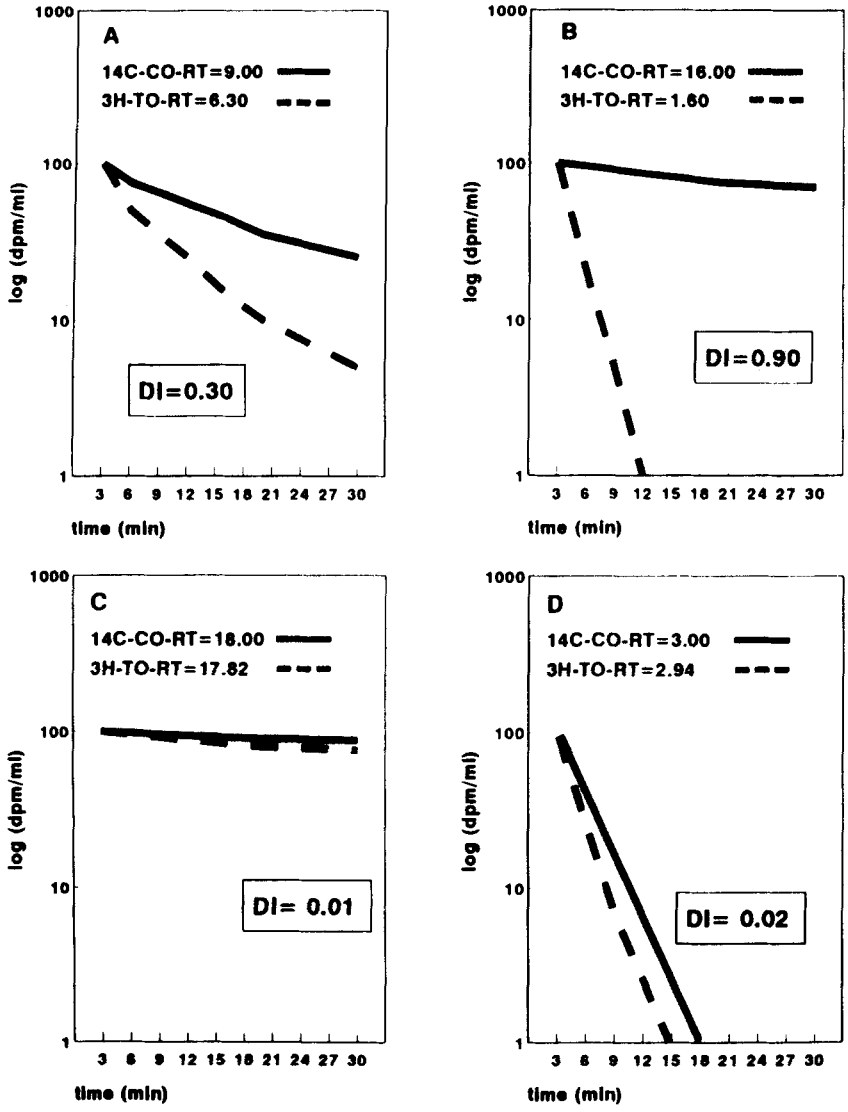

Fig. 1. Theoretical patterns of the metabolism of artificial chylomicrons infused intravenously, relating the residence times of [^{3}H]TO and [^{14}C]CO to the chylomicron delipidation rate. The delipidation index (DI) is expressed as $1 - \frac{[^3\text{H}]\text{TO-RT}}{[^{14}\text{C}]\text{CO-RT}}$ (the DI values presented are hypothetical). All of these patterns were actually found in experimental animals and in humans in the references cited. Normal subjects: panel A. Stimulation of lipoprotein lipase activity reduces [^{3}H]TO-RT, without necessarily reducing [^{14}C]CO-RT (13, 25) but markedly increasing the DI (25, 26); also, a poor recognition of the TG-rich particles by liver receptors brings on a longer [^{14}C]CO-RT and a far more complete loss of particle triglycerides characterized by a much shorter [^{3}H]TO-RT, namely very high DI values (26), panel B; lack of lipoprotein lipase activity (very low DI values) and consequently both remnant formation and uptake by the liver are exceedingly slow, as expected in Type I hyperchylomicronemias (25), panel C; intact triglyceride-rich particles are removed at a faster rate due to a larger number of receptors, greater receptor affinity, or diminished competition to the receptor from other lipoproteins, in spite of the low peripheral lipolysis (very low DI) due to a shorter exposure to lipoprotein lipase (26), panel D.

removal from plasma: the residence time of the particle remnant ([^{14}C]CO-RT) was significantly higher, as also reported by others (1, 14, 26). A typical familial hyperchylomicronemia case is presented in panel C, the primary defect being the absence of lipoprotein lipase activity (DI = 0.15) brought on by a markedly slow removal of the intact chylomicron particle. Furthermore, recent studies have indicated that attachment of lipoprotein lipase to the chylomicron is needed for its recognition by the liver receptors (34). Finally, a probably uncommon situation is presented in panel D: in one primary hypercholesterolemic subject the particle core is removed at a much faster rate than in normal subjects ([^{14}C]CO-RT = 3.85); much less time is available for interaction with lipoprotein lipase in peripheral tissues (DI = 0.11) because partially delipidated particles were taken up by the

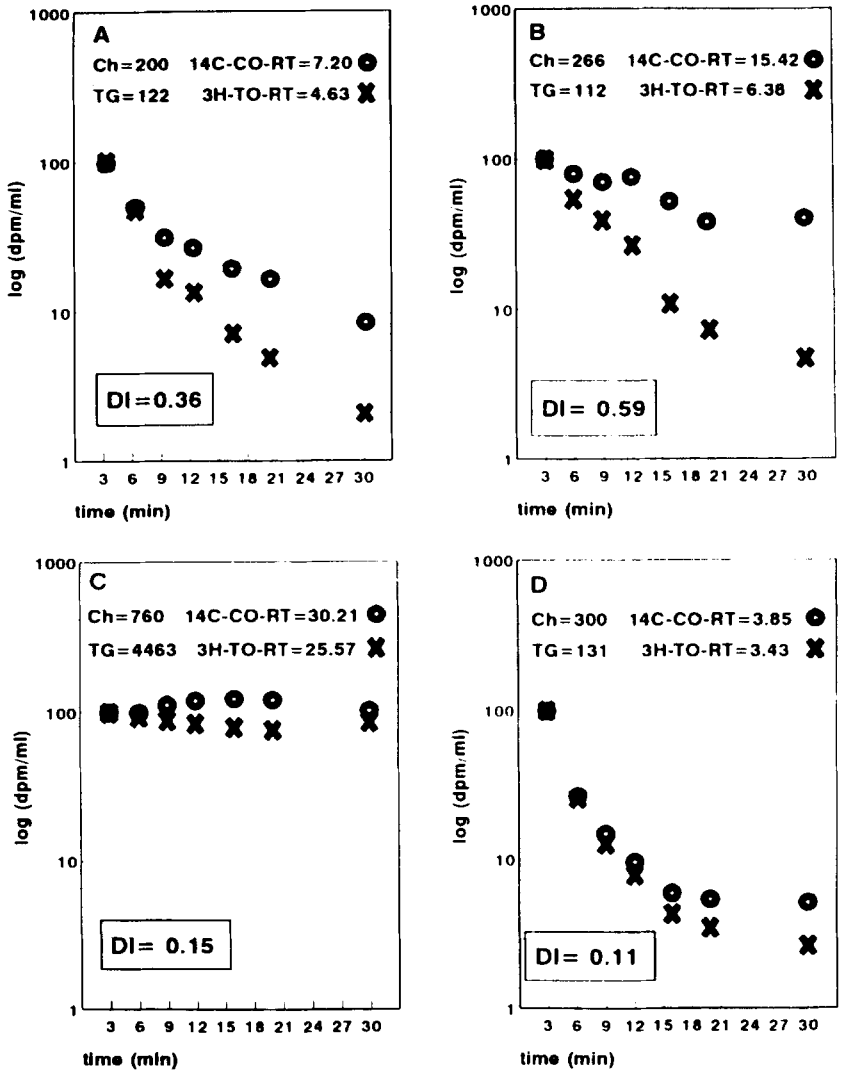

Fig. 2. Doubly labeled chylomicron emulsion containing [^{3}H]TO and [^{14}C]CO was infused into the blood stream of four representative subjects. The plasma concentrations of cholesterol (Ch) and triglyceride (Tg) are presented as mg/dl. Delipidation index (DI) 0.36, in a normolipidemic patient, panel A. A hypercholesterolemic hypothyroid patient presented a greater DI (0.59) probably secondary to a slower removal of the particle remnant ([^{14}C]CO-RT), panel B; severe hyperlipidemia due to the familial absence of lipoprotein lipase is compatible with a very low particle DI (0.15), panel C; a familial hypercholesterolemic subject removed the chylomicron particle at a higher rate with little time available for interaction with peripheral lipoprotein lipase (DI = 0.11): conceivably, there was little competition with VLDL-TG or LDL for splanchnic organ removal; this appears to be an unusual metabolic case (panel D).

liver at an increased rate, seemingly due to a much greater availability of lipoprotein receptors either owing to their greater numbers or as a consequence of less competition for uptake from other lipoproteins, such as LDL.

The parameters of artificial chylomicron metabolism are presented as a correlation matrix for normal subjects and primary hyperlipidemic patients (n = 39, **Table 2**). Plasma triglyceride concentration correlated with plasma cholesterol level, an expected finding due to the inclusion of primary mixed lipemia cases. Furthermore, as a consequence of correlations of the residence times of both [^{14}C]CO (r = 0.31843) and [^{3}H]TO (r = 0.44697) with the level of plasma triglycerides, the latter seems to reflect a slower rate of removal of triglyceride-rich particles. In this regard, the residence times of [^{3}H]TO and of [^{14}C]CO were most significantly correlated with each other (r = 0.87753) as predicted for processes representing rates of metabolism of the chylomicron remnants. The [^{3}H]TO decay curve simultaneously describes the rates of chylomicron delipidation and of splanchnic organ remnant uptake, whereas the [^{14}C]CO disappearance rate is due to remnant uptake alone. Obviously the correlation value would approach r = 1 as the delipidation index decreases. In the group as a whole, the level of plasma triglycerides

TABLE 2. Correlation matrix of plasma lipid concentration values and artificial chylomicron metabolic parameters in normal controls and primary hyperlipidemic patients (n = 39)

	Plasma		[^{14}C]CO RT[a]	[^{3}H]TO RT[a]
	Chol	TG		
Plasma TG	0.53986			
[^{14}C]CO RT	0.30944	0.31843		
[^{3}H]TO RT	0.29397	0.44697	0.87753	
DI[b]	0.03401	−0.33110	0.05045	−0.40877

Critical value = 0.31558 at $P < 0.05$. Significant correlations are underlined.
[a]RT, residence time of radioactivity in plasma.
[b]DI, delipidation index shown in Methods section.

was also reciprocally related to the degree of particle delipidation ($r = -0.33110$). The model also predicts that [^{3}H]TO-RT increases as the lipoprotein lipase-dependent particle lipolysis rate, represented by the delipidation index, is impaired ($r = -0.40877$). Furthermore, as indicated by other authors (13, 25–27), the extent of particle lipolysis by itself might not settle the rate of chylomicron remnant metabolism. In other words, the intensity of particle delipidation might, to a considerable extent, be independent of the rate of remnant uptake, which explains the lack of correlation between delipidation index and [^{14}C]CO-RT (0.05045).

Cases of severe primary hypertriglyceridemia due to combined hyperlipidemia, on the other hand, displayed a marked impairment of artificial chylomicron metabolism characterized by the long residence time of the remnant ([^{14}C]CO), which must be ascribed to the diminished lipolytic activity as shown by a much lower delipidation index and longer [^{3}H]TO residence time than that observed in control subjects (**Table 3**). This group of patients included one familial hyperchylomicronemia case in which radioactivity was not removed from plasma and thus the delipidation value was negligible.

DISCUSSION

Intravenous pulse infusion of doubly labeled artificial chylomicron emulsions is a novel and useful tool for elucidating the metabolic disorders in primary and secondary dyslipidemias as well as the mechanisms of action of drugs and diets that lower plasma triglyceride levels. Although radioactive material is used, such particles do not require retinyl palmitate or radioactive triglycerides alone to label chylomicron, as the latter procedures do not distinguish the different processes simultaneously involved in chylomicron metabolism, i.e., lipolysis and particle removal rates. The present method has the advantage over other procedures in that all steps of chylomicron metabolism are tracked through two simultaneous labeling devices: cholesteryl [^{14}C]oleate remains in the particle during the short span of the experiment (30 min) and thus represents solely the rate of particle core uptake by the splanchnic organs. [^{3}H]triolein chylomicron also does not appreciably exchange with other plasma lipoproteins, so that its plasma fall-off curve, faster than that of [^{14}C]cholesteryl ester, does represent a fraction of the chylomicron core that was lost from the particle as fatty acids before the

TABLE 3. Plasma residence times of [^{14}C]CO and [^{3}H]TO and delipidation index (DI)

Patients	Residence Time		DI
	[^{14}C]CO	[^{3}H]TO	
	min, mean ± SD		
Controls	9.52 ± 4.33	5.87 ± 1.76	0.33 ± 0.16
Hypercholesterolemia (primary)	11.15 ± 6.51	6.64 ± 4.83	0.38 ± 0.18
Combined hyperlipidemia (moderate)	10.81 ± 4.94	7.23 ± 3.45	0.30 ± 0.14
Combined hyperlipidemia (severe)	17.83 ± 7.68[a]	14.76 ± 6.64[b]	0.17 ± 0.09[a]

Statistical comparisons between groups were performed by ANOVA.
[a]$P < 0.05$, when compared to controls.
[b]$P < 0.01$, when compared to controls.

particle was picked up by the visceral organs. Consequently, the plasma $^{3}H/^{14}C$ ratio represents the amount of [^{3}H]triolein present in the intact particle that is captured by the organs and 1 – $^{3}H/^{14}C$ is the fraction of the chylomicron [^{3}H]triolein shed off by hydrolysis by lipoprotein lipase. Also, the plasma retinyl palmitate curve obtained after oral administration of [^{3}H]retinol requires plasma ultracentrifugation for separation of chylomicrons from their remnants (2–7, 11, 12) and from the triglyceride-rich lipoproteins originating from the liver during alimentary lipemia (35–37). Finally, available data on the retinyl ester chylomicron kinetics pulse infused intravenously during the fasting period (11–13) cannot strictly be compared to those drawn from the plasma retinyl ester tolerance curve simultaneous to the intake of retinol and of fat (2–10).

In previously described moderate hypertriglyceridemic subjects (3, 4), as well as in normotriglyceridemic subjects who differed in apoE lipoprotein phenotype (1, 7, 11), plasma retinyl ester tolerance curves disclosed delayed clearances both of the plasma chylomicrons and of their remnants. However, these data are not strictly comparable to ours because artificial chylomicron emulsions were infused in the fasting state, whereas the plasma retinyl ester curves resulted from an oral fat tolerance test containing retinol. Meal absorption stimulates the production of VLDL-triglycerides that must compete for removal with chylomicrons (38, 39). Therefore, the artificial chylomicron administration test during the alimentary phase is needed in order to compare the sensitivity of the two methods in identifying partial hindrance of chylomicron metabolism.

The artificial chylomicron can also be labeled with radioactive retinyl ester instead of cholesteryl ester. However, the retinyl ester molecule is highly susceptible to oxidation and thus difficult to handle in the preparation and administration of chylomicrons. In one study on dogs, the plasma radioactive decay curves of natural chylomicrons were similar for cholesteryl oleate and retinyl ester (40). In another study on rabbits, however, 25 min after the chylomicron intravenous pulse infusion, the plasma disappearance of cholesteryl [^{14}C]oleate became faster than that of [^{3}H]retinyl ester (35). Nonetheless, this study was flawed by the fact that the natural chylomicrons infused contained only 2% of radioactive free retinol as compared to 15% of unesterified cholesterol, which exchanges quite rapidly among plasma lipoproteins.

Artificial chylomicron kinetics in rats had monoexponential decay curves in plasma for both [^{3}H]triolein and cholesteryl [^{14}C]oleate (23–26). However, in human studies both kinetics fitted multiexponential curves, so that plasma residence times were obtained. In our normal subjects, the mean RT in plasma was compared to data from other studies in which the half-life values or fractional catabolic rates (FCR) were transformed into RT values, respectively calculated as $T_{1/2}/\ln 2$ and as 1/FCR. Mean RT (min) data for normal subjects after intravenous pulse infusion of chylomicrons containing retinyl ester were 27.1 (20) and 27.0 (11), and individual values were 14.4, 8.2, and 12.5 in another study (13). Our remnant data ([^{14}C]CO-RT = 9.52 min) were much smaller than in the studies mentioned where a considerable plasma volume containing triglyceride was infused intravenously; however, the retinyl ester removal rates have been shown to be reciprocally related to the amount of plasma triglyceride infused (12). On the other hand, our mean [^{3}H]triolein RT in normal subjects was 5.8 min which is close to the plasma triglyceride residence times obtained after steady duodenal triglyceride perfusion (6.5 min, refs. 16 and 17), and the 7.2 min to 11.5 min range drawn from the intravenous kinetic data of autologous radioactive chylomicrons that had been labeled after the oral intake of [^{14}C]palmitic acid (20). However, our mean [^{3}H]TO-RT was considerably shorter than the values obtained by the intravenous bolus infusion of Intralipid® (19.7 min, ref. 17; and 16.4 and 13.9 min, ref. 18). Nonetheless, the latter authors (18) administered 0.05 to 0.1g of fat/kg body weight which might also have a mass effect on the radioactive chylomicron removal rate. Finally, in one study, unlabeled chylomicrons drawn from the thoracic duct were pulse-infused intravenously into two normal subjects and the plasma RT was about 7.6 min (19) which is comparable to our data on [^{3}H]triolein removal.

Except for apoB lipoprotein, artificial chylomicrons gain plasma apolipoproteins when injected into the blood stream (23). In spite of the absence of apoB-LP, the rates of removal of artificial emulsions are quite similar to those of natural chylomicrons in animal models (29) and in human studies (16–20). Experimental data in rats strongly suggest that the largest share of chylomicrons is taken up by liver receptors that are independent of the apoB-LP-recognizing receptors (41–43). In this regard, chylomicron uptake was not disturbed in human primary hypercholesterolemia due to a B/E receptor defect as shown in leukocytes (44) and hepatoma cells (45) in previous in vitro and in vivo studies (4, 8, 15), and in primary hypercholesterolemic subjects in the present report. In spite of biochemical evidence that the human liver LDL receptor recognizes chylomicron remnants (46), chylomicron metabolism impairment did not occur in the Watanabe-heritable-hyperlipidemic rabbit (43), although partial hindrance has recently been suggested in these genetic hypercholesterolemic animals which, incidentally, were investigated by the technique of infusing chylomicron remnants devoid of apoB-containing lipoproteins (47). This issue, nonetheless, must be clarified in experiments of VLDL kinetics in familial hypercholesterolemic subjects as, differently from chylomicrons, the VLDL-

remnant clearance may be reduced during the alimentary state in these patients (15).

In some primary hypercholesterolemic patients, a population of LDL with slow plasma clearance attributed to defective LDL was described (48). This fact raises the possibility that the turnover of triglyceride-rich lipoproteins might be accelerated due to less competition for the LDL-receptor that might have occurred in the moderate primary hypercholesterolemic subject shown in Fig. 2, panel D, where the chylomicron removal rate was unusually faster.

Moderate hypertriglyceridemia must be ascribed to high levels of plasma VLDL-TG because chylomicrons were normally metabolized by lipoprotein lipase, as shown by delipidation index values close to those of normal controls. In these patients VLDL-TG concentration could then be secondary to poor VLDL recognition by specific liver B/E receptors or, additionally, to combinations of variable degrees of VLDL-TG overproduction by the liver (1, 49). Thus, mild plasma elevations of VLDL-TG might occur by mechanisms other than defects in chylomicron metabolism. On the other hand, although the artificial chylomicron metabolic parameters are not significantly disturbed in moderate hypertriglyceridemic subjects when compared to normotriglyceridemic subjects (Table 3), the correlation coefficient matrix (Table 2) clearly relates the plasma triglyceride concentration range to an impairment of the delipidation process ($r = -0.3311$) which elicits a slower chylomicron particle removal rate (longer [^{3}H]TO and [^{14}C]CO residence times). Consequently, patients with distinctly elevated plasma triglyceride levels present slower particle lipolysis rates than indicated by others (50). The latter bring on accumulation of chylomicron remnants in the fasting state together with intact VLDL and probably VLDL remnants that normally seem to appear in plasma only during the alimentary period (12). If the primary metabolic defect had been solely an impairment of remnant particle uptake, then a more complete or efficient lipolysis, namely a higher delipidation index, would ensue as indicated in experimental animal studies (25, 26) and as suggested in some hypothyroid cases such as the one presented in Fig. 2, panel B. Insufficiently delipidated chylomicrons and VLDL alike may be poorly recognized by the liver receptors for these particles.

This study sheds light on the complex metabolic interrelationships between endogenous and exogenous triglyceride-rich particles and on factors that potentially regulate the metabolism of these lipoproteins, such as the activity of lipoprotein lipase, in the control of the chylomicron remnant removal from plasma. The use of doubly labeled artificial chylomicrons demonstrates, for the first time, that in normal and in hypercholesterolemic subjects roughly 17–58% of the particle triglyceride content sheds off in plasma before the remnants are taken up by splanchnic organs and that this fraction is considerably smaller (8–26%) in severe hypertriglyceridemia. The liver, most likely, has to cope with the largest share of the exogenous fat that must be metabolized into acetate or re-excreted in blood as VLDL-TG. This possibility is compatible with a recent study showing that a fat meal elicits a greater rise in the plasma concentration of apoB-100, a marker of VLDL, than in apoB-48, which is a marker of chylomicrons, and that variation in VLDL is responsible for about 50% of the increment in plasma triglycerides (51).

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