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Relationship between Plasma Triglycerides and Removal of Chylomicrons *

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The hypertriglyceridemia that follows the ingestion of fat is significantly greater throughout the period of absorption in patients with coronary heart disease (CHD) than in normal subjects (1, 2). Since in fasting subjects the levels of plasma triglycerides are also abnormally elevated in patients with CHD (3, 4), it has been suggested that the prolonged alimentary lipemia might be related to the presence of an abnormally large pool of endogenous triglyceride in the liver and plasma (5, 2). This has been supported by Denborough's finding of a close correlation between the degree of alimentary lipemia and the fasting plasma triglyceride levels; reduction of the fasting triglyceride level is accompanied by a proportional diminution in postprandial hypertriglyceridemia. Additional support is derived from our previous demonstration that, provided the concentrations of plasma lipids are normal, the rates of removal from the blood of intravenously administered human lymph chylomicrons are, on the average, similar in subjects with and without CHD (5). An alternative suggestion is that defective intestinal handling of fat might lead to abnormal alimentary lipemia (6).

In the same group of patients at different triglyceride concentrations, I have investigated the relationships between the fasting triglyceride concentration, the intensity of the lipemia that follows a fatty meal, and the rate at which chylomicrons leave the circulation. The rates of removal of intravenously administered lymph chylomicrons and orally administered fat have been compared in twelve subjects with and without CHD whose plasma triglyceride concentrations varied from 36 to 339 mg per 100 ml.

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Methods

Intravenous administration of chylomicrons. Human lymph chylomicrons were obtained from a subject with carcinoma of the breast whose thoracic duct had been cannulated at the time of a lymph node biopsy. On the following day the patient consumed a meal containing 70 g of fat, together with about 2 mc of palmitic acid—9,10-H_3,[1] and the chyle was collected under aseptic conditions. Chylomicrons were separated by spinning the chyle under saline in sterile containers at a density of 1.006 in the 30 rotor of a Spinco model L ultracentrifuge at 20,000 rpm for 1 hour at 4° C. The creamy supernatant layer was resuspended in 0.9% NaCl and stored at 4° C. The chylomicrons were checked for sterility before use. The infusion experiments were completed within 2 weeks of the collection of the chyle.

Fractions of this chylomicron preparation were infused into eight males with CHD, ages 37 to 49 (mean, 44), to be referred to as patients, and four healthy males, ages 32 to 53 (mean, 41), to be referred to as controls. The patients with CHD had survived at least one myocardial infarction within the preceding 2 years but had returned to work by the time of the study. They were not receiving any drugs and were not on diets. The control subjects were fully ambulant men attending the hospital for minor investigations. After the subjects had fasted overnight, an indwelling catheter was inserted into a vein for the collection of blood. The chylomicrons were briefly warmed at 37° C to ensure dispersion (confirmed microscopically) and then rapidly infused into a vein of the other arm. The amount of triglyceride infused was related to the subject's body weight; on the average, 1.05 g of triglyceride and 30 μc of tritium were administered.

Blood was collected into chilled, heparinized tubes 2, 5, 10, 15, 20, 40, and 60 minutes after the infusion. The plasma was separated (2,000 *g* for 10 minutes) and then centrifuged under 0.9% NaCl at 30,000 rpm for 1 hour in the 40.3 rotor of the ultracentrifuge at 4° C. The top layer of chylomicrons was removed by a slicer and the lipids extracted into chloroform: methanol (2:1, vol: vol). After the addition of $\frac{1}{5}$ vol of 0.02 N HCl, the lipids contained in the chloroform phase were separated on columns of silicic acid into neutral lipids and phospholipids by eluting with chloroform and methanol re-

[1] Nuclear Chicago, Des Plaines, Ill.

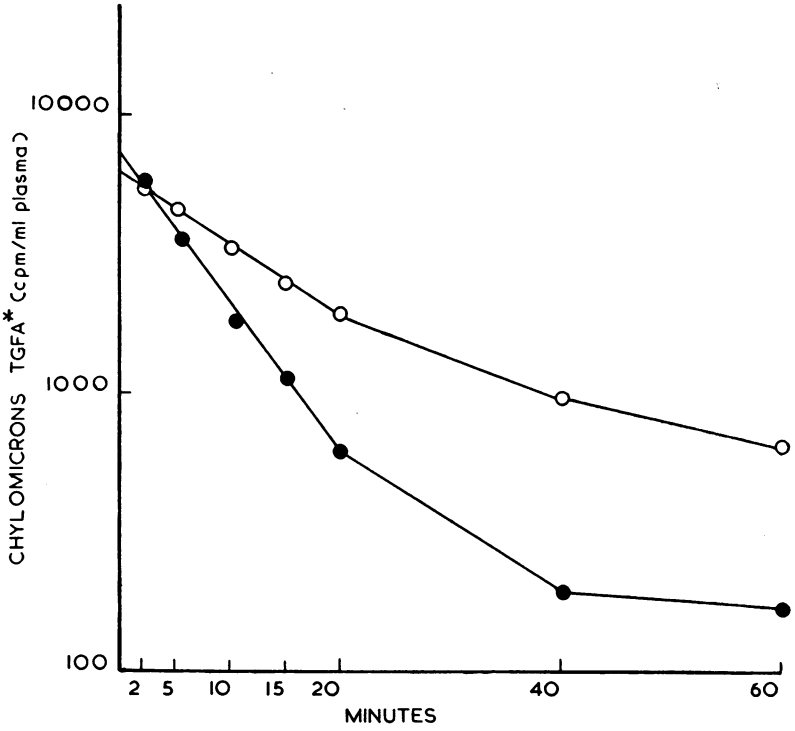

FIG. 1. RATE OF REMOVAL FROM THE BLOOD OF RADIOACTIVITY ASSOCIATED WITH TRIGLYCERIDE OF CHYLOMICRONS IN TWO PATIENTS WITH CORONARY HEART DISEASE (● = Patient 5; ○ = Patient 9; TGFA = triglyceride fatty acids.)

spectively (7). Radioactivity in the neutral lipid and phospholipid fractions was assayed in a liquid scintillation counter, with 0.3% diphenyloxazole in toluene as scintillator solvent.

After the removal of the layer of chylomicrons, the infranatant layers of the 10-, 20-, 40-, and 60-minute samples of the first seven experiments were centrifuged at a density of 1.006 for a further 16 hours at 100,000 × *g*. The lipoproteins at the bottom of the tubes were extracted into chloroform: methanol. The lipids were separated on silicic acid columns into fractions containing cholesterol esters (by eluting with 1% diethyl ether in heptane), glycerides, free fatty acids and free cholesterol, and phospholipids. Free fatty acids were separated from glycerides by the method of Borgström (8) and titrated by Dole's method (9). Radioactivity was determined in free fatty acids, triglycerides, cholesterol esters, and phospholipids.

Studies of alimentary lipemia. At the conclusion of the iv studies, eleven of the twelve subjects consumed a 70-g fat meal consisting of eggs, milk, cream, butter, and bread and identical in composition to the meal eaten by the donor of the chylomicrons. Samples of blood were obtained 3, 5, and 7 hours after the meal; triglyceride concentrations were measured on samples of the plasma by the method of van Handel and Zilversmit (10).

Results

Intravenous administration of chylomicrons. The rate of removal of the radioactivity in neutral lipids [2] of the chylomicron fractions of plasma followed an exponential course for the first 15 to 20 minutes (Figure 1, Table I). Thereafter the rate of removal proceeded at a series of slower rates. The $t_{\frac{1}{2}}$ of removal were calculated from the linear portion of the slope.

The $t_{\frac{1}{2}}$ of removal varied from 5.0 to 13.0 minutes (Table I) and was significantly related to the fasting plasma triglyceride concentration ($p = < 0.001$; $r = + 0.93$; Figure 2).

Although the mean rate of removal was significantly faster for the control than the patient group ($p = < 0.05$), the rates of removal in the two pa-

[2] Further separation of neutral lipids in eight samples of plasma revealed that 95% of the radioactivity was present in the triglyceride fraction.

TABLE I

Fasting plasma triglyceride concentrations and rates of removal of radioactivity in the neutral lipids of intravenously administered lymph chylomicrons

Subjects	Fasting plasma triglyceride concentration	Radioactivity in neutral lipids of chylomicrons (Minutes)							$t_{\frac{1}{2}}$ of removal of radioactivity
		2	5	10	15	20	40	60	
	mg/100 ml	*cpm/ml plasma*							*minutes*
Normal controls									
1	36	6,060	4,110	1,910	1,050	570	320	190	5.0
2	90	5,210	4,090	2,510	1,720	1,150	750	480	8.0
3	92	4,950	3,550	2,400	1,420	950	810	690	7.0
4	93	5,810	4,010	2,090	1,110	Lost	300	190	5.5
Coronary heart patients									
5	79	5,750	3,810	1,890	1,060	610	190	170	5.0
6	128	5,170	3,600	2,110	1,110	630	300	270	5.5
7	174	5,560	4,610	3,040	1,950	1,400	Lost	610	8.5
8	175	4,950	4,100	2,610	1,810	1,300	920	690	9.0
9	199	5,500	4,590	3,220	2,310	1,680	950	650	10.0
10	215	5,510	5,080	3,510	2,320	1,730	1,070	620	10.0
11	288	4,950	4,400	3,370	2,510	1,950	980	710	12.5
12	339	4,750	4,100	3,180	2,340	1,900	990	790	13.0

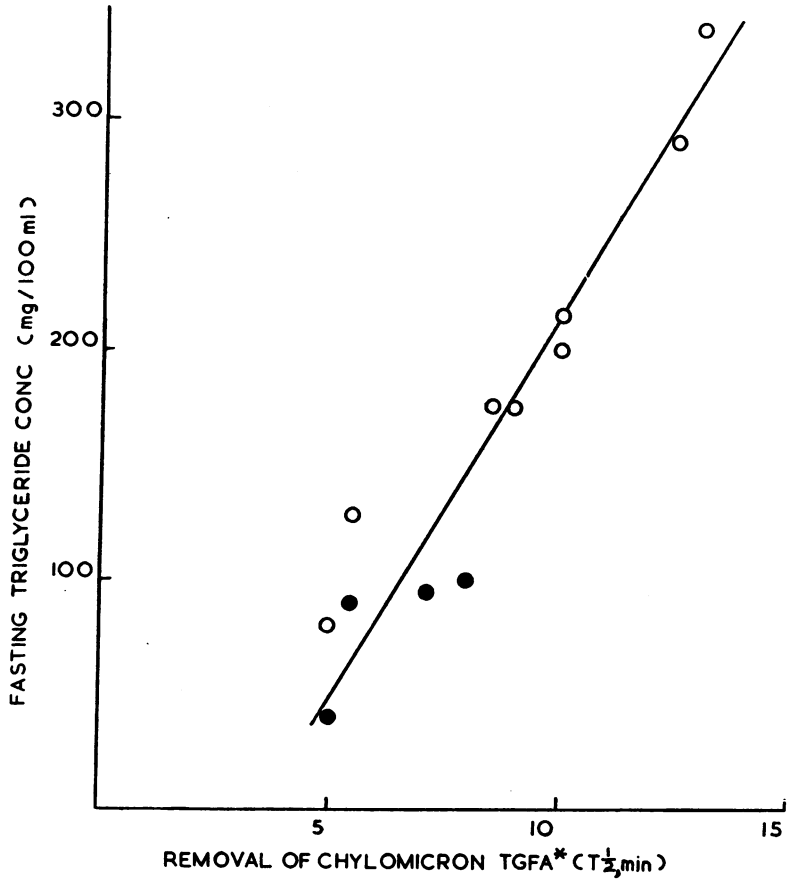

FIG. 2. RELATIONSHIP BETWEEN FASTING PLASMA TRIGLYCERIDE CONCENTRATIONS AND THE $T_{\frac{1}{2}}$ OF REMOVAL OF INTRAVENOUSLY ADMINISTERED LYMPH CHYLOMICRONS (○ = patients; ● = controls).

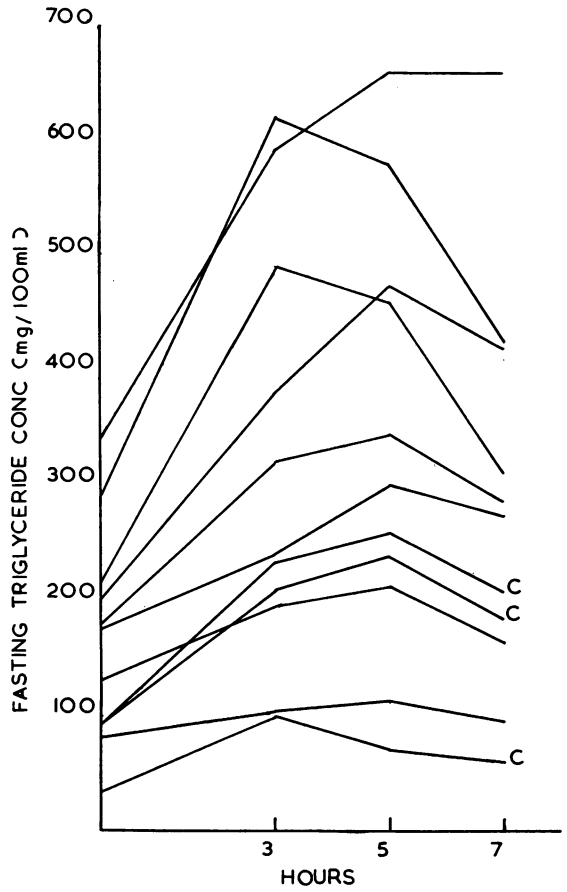

FIG. 3. PLASMA TRIGLYCERIDE LEVELS 3, 5, AND 7 HOURS AFTER THE INGESTION OF A 70-G FAT MEAL IN EIGHT SUBJECTS WITH CORONARY HEART DISEASE AND IN THREE CONTROL SUBJECTS (C).

tients with normal plasma triglyceride concentrations [3] were among the fastest (Table I, Patients 5 and 6).

Radioactivity associated with the phospholipids of the chylomicrons accounted for about 5% of the total radioactivity. Its rate of removal cannot be assessed, since considerable exchange of radioactivity occurred between the phospholipids of chylomicrons and other lipoproteins.

Radioactivity in other lipoproteins. The radioactivity found at any one time in the free fatty acids and in the lipids of lipoproteins of density greater than 1.006 was generally about 2 to 6% of the injected radioactivity. The largest fraction of this radioactivity was found in the free fatty acids. The rate of rise of free fatty acid specific activity was directly related to the rate of removal of chylomicrons; e.g., in the two patients in whom the $t_{\frac{1}{2}}$ of removal of chylomicron triglycerides were 5 and 13 minutes, the respective peak free fatty acid specific activities were reached at 20 and 60 minutes. Eventually, however, the peak free fatty acid specific activity was similar in most patients and was equivalent to slightly less than 5% of the chylomicron triglyceride fatty acid specific activity. Only minimal amounts of radioactivity were found in the triglycerides of lipoproteins with a density greater than 1.006.

[3] I have found the mean fasting plasma triglyceride concentration in 51 middle-aged healthy men to be 120.3 ± 55.0 mg per 100 ml.

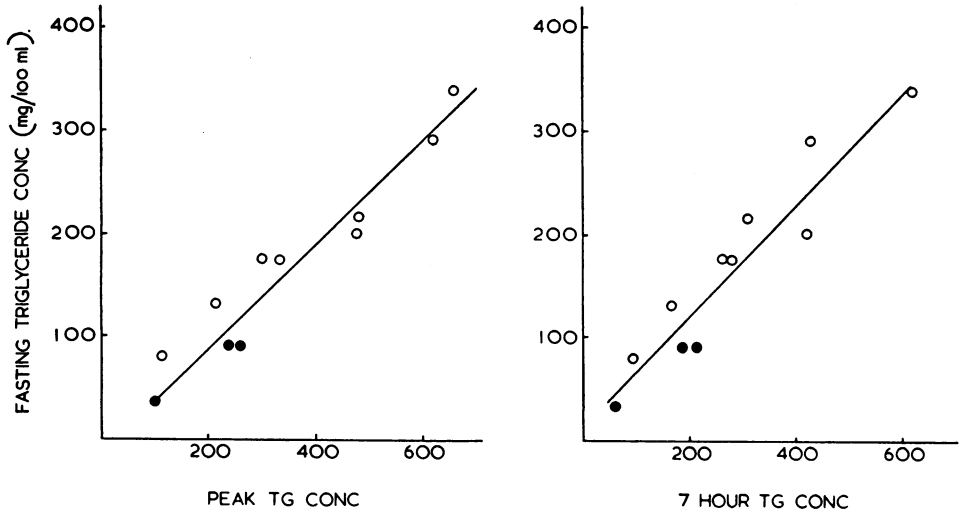

FIG. 4. RELATIONSHIP BETWEEN THE FASTING PLASMA TRIGLYCERIDE CONCENTRATIONS AND THE TRIGLYCERIDE CONCENTRATIONS AT PEAK LEVELS AND AT 7 HOURS AFTER THE INGESTION OF A 70-G FAT MEAL (○ = patients; ● = controls).

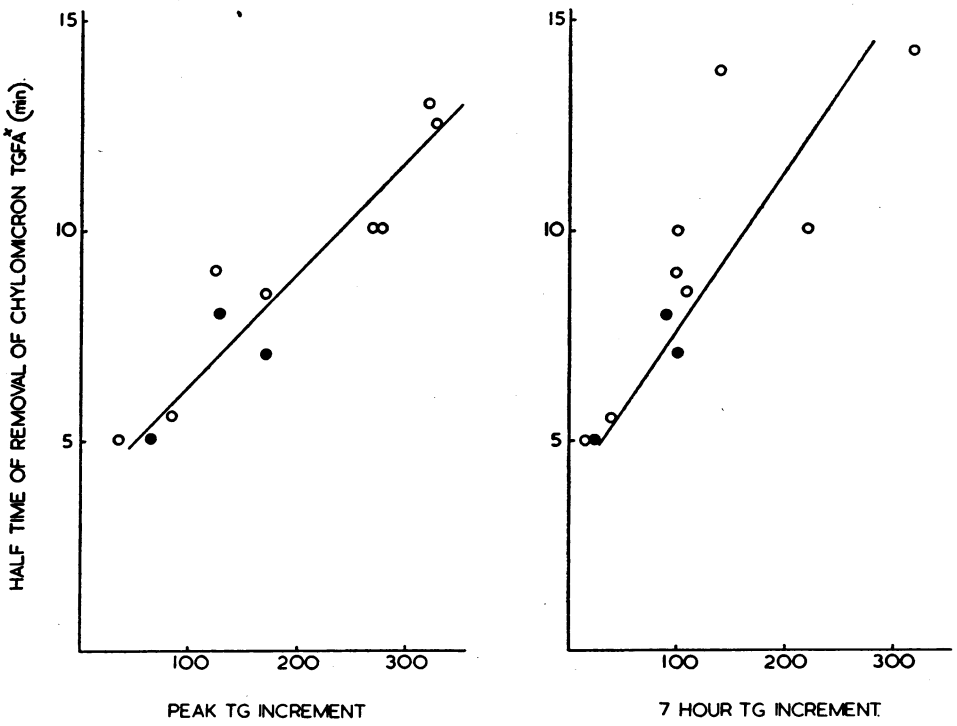

FIG. 5. RELATIONSHIP BETWEEN THE $T_{\frac{1}{2}}$ OF REMOVAL OF INTRAVENOUSLY ADMINISTERED LYMPH CHYLOMICRONS GIVEN IN THE FASTING STATE AND THE INCREMENTS IN PLASMA TRIGLYCERIDE CONCENTRATIONS AT PEAK LEVELS AND AT 7 HOURS AFTER THE INGESTION OF 70-G FAT MEALS (○ = patients; ● = controls).

About 10% of the injected radioactivity was present in cholesterol esters and phospholipids of the chylomicrons; approximately one-third of this radioactivity was later found in the corresponding fractions in higher density lipoproteins, probably due to exchange.

Studies of alimentary lipemia. In general, triglyceride concentration was maximal 5 hours after the ingestion of the 70-g fat meal (Figure 3). The degree of postprandial hypertriglyceridemia was related to the fasting plasma triglyceride concentration. Both the peak and 7-hour triglyceride concentrations were significantly related to the fasting level ($p = < 0.001$ in both comparisons; $r = + 0.93$ and $+ 0.95$; Figure 4).

A measure of the intensity of an alimentary lipemia is the difference between the fasting and peak triglyceride concentrations. Similarly, the difference between the fasting and 7-hour triglyceride concentrations is an index of clearing of the ingested fat. There was a highly significant relationship between the rate of removal of the intravenously administered triglycerides in the lymph chylomicrons ($t_{\frac{1}{2}}$) and the intensity and the clearing of the alimentary lipemia as defined above ($p = < 0.001$ for both comparisons; $r = + 0.93$ and $+ 0.84$; Figure 5).

The intensity of alimentary lipemia was greater and the clearing of the lipemia slower in the patient group than in the control group (Figure 3), but due to the small number of controls the difference was statistically not significant. There was, however, no apparent difference among those patients and controls whose fasting triglyceride levels were normal.

Discussion

The removal of radioactivity associated with the neutral lipids of chylomicrons was exponential during the first 20 minutes. This is consistent with previous studies in dogs (11, 12) and in man (5, 13). The subsequent removal was more complex and would be consonant with the simultaneous removal of several populations of chylomicrons

proceeding at different rates. It might also indicate different rates of removal at different sites. Recirculation of injected radioactivity reappearing in very low density lipoproteins might be an additional factor, but similar studies in dogs have revealed only minimal recirculation of label during the first 20 minutes (14). The extent of exchange either *in vivo* or during the brief centrifugation *in vitro* did not appear to be significant, since less than 2% of radioactivity present in the plasma at any given time was found in the triglycerides of lipoproteins of density greater than 1.006. This is the order of magnitude described previously in *in vivo* studies with dogs (12).

The results demonstrate the existence of a close interrelationship between the fasting plasma triglyceride concentration, the intensity of the lipemia after a fatty meal, and the rate at which chylomicrons leave the circulation (Figures 2, 4, and 5). Both the intensity and duration of alimentary lipemia and the rate of removal of intravenously administered chylomicrons from the blood have been shown to be directly related to the fasting triglyceride concentration. This implies that they are also related to the size of the pool of triglycerides contained within the liver and plasma, since in rabbits the plasma triglycerides, especially those carried in very low density lipoproteins, are in equilibrium with those in the liver (7). Such a deduction appears reasonable when considered in the context of chylomicron metabolism. Chylomicrons are in part removed in the liver, where the triglycerides of exogenous and endogenous (hepatic) origin reach isotopic equilibrium (14). The triglycerides are subsequently discharged from the liver into the plasma in the form of very low density lipoproteins and are eventually taken up by extrahepatic tissues together with the triglycerides of that fraction of chylomicrons that are not removed in the liver (15). Since both the chylomicron triglycerides and the triglycerides of endogenous origin share many common metabolic pathways in the liver and plasma, the rate at which chylomicrons are cleared from the blood will be influenced by the magnitude of the liver-plasma pool of triglycerides. The fractional turnover rate of a substance is inversely related to the total quantity within the pool, which is consistent with the finding that the rate of removal of chylomicrons was slowest in subjects with elevated plasma triglyceride concentrations and vice versa.

This explanation appears adequate for the frequent observations that postprandial plasma triglycerides are higher in patients with coronary heart disease (1, 2), since such patients frequently have elevated plasma triglyceride concentrations (3, 4). This suggests that abnormal alimentary absorption need not be postulated (6).

My findings also indicate that removal of intravenously injected chylomicrons is normal in those patients with coronary heart disease whose plasma triglyceride concentrations are normal. This is in agreement with our previous observations (5).

These findings do not rule out the possibility that defective removal mechanisms might be a factor in producing hypertriglyceridemia, since diminished removal of triglycerides, by extrahepatic tissues for instance, would delay the removal of endogenous triglycerides as well. Should the rate of production of endogenous triglycerides exceed their rate of removal, hypertriglyceridemia would result, a possibility suggested by the demonstration, in hypertriglyceridemic patients, of an abnormally increased flux of free fatty acids in response to norepinephrine (16).

The recycling of free fatty acids after the removal of chylomicrons was of a similar order of magnitude to that found in the dog (11). Although the rate of influx of labeled free fatty acids into the plasma depended on the rate of chylomicron removal, the magnitude of this recycling was similar in all subjects, indicating similar mechanisms of removal.

Summary

The relationships among the intensity of an alimentary lipemia, the rate of removal of chylomicrons from the blood, and the fasting plasma triglyceride concentration were studied in eight patients with coronary heart disease and in four control subjects.

Human lymph chylomicrons, isotopically labeled *in vivo,* were administered intravenously. The rate of removal from the blood was inversely related to the fasting plasma triglyceride concentration, and the correlation was highly significant.

The peak and 7-hour plasma triglyceride concentrations after the ingestion of a 70-g fat meal were directly and significantly related to the fasting triglyceride levels. The increments in plasma triglyceride concentration after these meals were significantly related to the $t_{\frac{1}{2}}$ of removal of intravenously administered lymph chylomicrons.

These findings apply equally to patients with and without coronary heart disease.

I conclude that the intensity of an alimentary lipemia is related to the size of the liver-plasma pool of triglyceride, which determines the rate of removal of chylomicrons from the blood. The greater size of this triglyceride pool in patients with coronary heart disease is probably responsible for the intense alimentary lipemia that these patients frequently develop.

Acknowledgments

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