Continuous intravenous heparin administration in humans causes a decrease in serum lipolytic activity and accumulation of chylomicrons in circulation

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Abstract Heparin is a well-known, widely used anticoagulant drug. In addition to its anticoagulant properties, however, it also has a marked influence on fat metabolism. Postprandial lipoproteins may contribute significantly to the development of coronary heart disease. Therefore, it is important to evaluate the effects of heparin on these lipoproteins. The effect of continuous heparin administration on postprandial lipoprotein metabolism was studied in 11 patients with thromboembolic disease. Results were compared with those in a group of six patients given no heparin. Two vitamin A-fat loading tests were done: the first, 5 days before heparin was started and the second, on the fourth day of continuous heparin drip of 1000 U/h, maintaining PTT levels at twice the baseline. To study the effect of acute heparin, an additional fat loading test was done in five patients on the first day of heparin treatment. Vitamin A specifically labels intestinally derived lipoproteins with retinyl palmitate (RP). The concentrations of chylomicron (S_f>1000)- and nonchylomicron (S_f<1000)-retinyl palmitate were measured for 10 h postprandially. Four days of continuous intravenous heparin administration increased the area below the chylomicron RP curve from 11091 ± 4393 to 17684 ± 5949 μg/l · h (P <0.003). When measured on the first day of heparin treatment in five patients, the area of the chylomicron fraction was reduced from 16678 ± 6895 to 10474 ± 3893 μg/l · h (P < 0.05). Postheparin lipoprotein lipase activity was significantly lower on the fourth day of heparin administration than before treatment: 1.8 ± 1.1 vs. 4.1 ± 1.3 μmol/FFA per ml per h, respectively (P < 0.0005). In the six control patients with thromboembolic disease in whom heparin therapy was not indicated, no changes in postprandial lipoprotein levels or in lipolytic activity during hospitalization were found. The study demonstrates that 4 days of heparin administration causes an accumulation of chylomicrons in the circulation, most probably as a result of a marked decrease in serum lipolytic activity.—**Weintraub, M., T. Rassin, S. Eisenberg, Y. Ringel, I. Grosskopf, A. Iaina, G. Charach, M. Liron, and A. Rubinstein.** Continuous intravenous heparin administration in humans causes a decrease in serum lipolytic activity and accumulation of chylomicrons in circulation. *J. Lipid Res.* 1994. **35:** 229–238.

Supplementary key words heparin • serum lipolytic activity • chylomicrons

Heparin is a well known anticoagulant drug, widely used in the treatment of acute thromboembolic disease or as a preventive measure in high risk patients (1–4). In addition to its anticoagulant properties, heparin also has a marked influence on fat metabolism. For example, an intravenous injection of heparin results in immediate clearance of alimentary lipemia (5). This clearing action is due to the release of at least two hydrolytic enzymes, lipoprotein lipase (LPL) and hepatic triglyceride hydrolase (HTGL), from their normal location on capillary walls into the circulation (6–9).

LPL is synthesized within subendothelial cells and secreted by unknown routes to its site of action at the capillary endothelium, to which the enzyme is anchored by a glycosaminoglycan, probably heparan sulfate (10–13). LPL is responsible for the intravascular hydrolysis of plasma triglycerides. The second key enzyme, HTGL, is synthesized in the liver and secreted to its site of action on the sinusoidal surfaces of hepatic endothelium cells, where it is anchored in a fashion similar to that of LPL. The metabolic role of HTGL is controversial. It has been suggested that its main function is to break down high density lipoproteins (HDL) (14). It may also be important in VLDL remnant and chylomicron remnant metabolism (15–21).

Abbreviations: RP, retinyl palmitate; LPL, lipoprotein lipase; HTGL, hepatic triglyceride hydrolase; HDL, high density lipoprotein; VLDL, very low density lipoprotein; HPLC, high performance liquid chromatography; PTT, partial thromboplastin time.

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Although the enzymatic and metabolic effects of acute high-dose heparin injection have been studied in depth, little is known about the long-term effects of heparin administration on the metabolism of the postprandial lipoproteins (chylomicrons and their remnants). It has been speculated that repeated heparinization during chronic hemodialysis is associated with the decreased plasma triglyceride clearance rate and hypertriglyceridemia found in patients with chronic renal failure (22–24). A decrease in plasma intralipid clearance rate was also found in patients with normal kidney function who were on prolonged anticoagulant therapy with heparin for prophylaxis of thromboembolic disease (25). However, no difference has been noted in fat-removal capacity between heparin-treated and nonheparin-treated intensive care patients (26). In chronically heparinized animals, fasting serum concentrations of triglycerides were unaffected, but exogenous ^{14}C-labeled VLDL showed accelerated clearance rates (27).

Postprandial lipoproteins may be atherogenic and may contribute significantly to the development of coronary heart disease (28–30). As heparin is a widely used drug, it is of importance to evaluate its effects on these lipoproteins. In the present study we used the vitamin A-fat loading test, which specifically labels chylomicrons and chylomicron remnants with retinyl palmitate (15, 31–35), in order to investigate the effects of acute and chronic heparin treatment on postprandial lipoprotein metabolism in humans.

PATIENTS AND METHODS

Patients

The study population included 17 patients (12 males, 5 females; mean age, 71 ± 14 years; age range, 31–80 years) hospitalized for mild brain ischemia. All underwent brain CT and echocardiography. Eleven patients, in whom a thromboembolic complication of heart disease was confirmed or highly suspected and brain bleeding was excluded, were placed on heparin treatment (study group). In another six patients, no indication for heparin treatment was found (control group). Patients were in stable cardiovascular and metabolic condition at the time of testing, and continued any medication they were on at the time: beta-blockers (6 patients), digitalis (8 patients), nitrates (10 patients), and calcium antagonists (9 patients). Exclusion criteria were past history or presence of congestive heart failure, diabetes, liver, kidney or other metabolic–endocrinologic disease. Informed consent was obtained in all cases.

Study design

Study group (**Fig. 1**). Anticoagulant heparin therapy was started in the study group (11 patients) about 10 days after the acute event. An intravenous bolus of 5000 U was followed by a continuous drip of 1000 U/h to maintain PTT values at twice the baseline. The vitamin A-fat loading test was performed 2–5 days before onset of heparin treatment and on day 4 of treatment. Five patients underwent a fat loading test also on day 1 of heparin treatment. In the latter test, the bolus of 5000 U was administered 2 h after the meal was eaten, followed by the drip as above. Postheparin lipolytic activities were measured twice: first, before heparin therapy was started and second, on the fourth day of heparin administration.

Control group. The six patients not treated with heparin underwent a vitamin A-fat loading test and a post-heparin lipoprotein lipase test at the beginning of hospitalization (days 3–4) and before discharge (days 12–14).

Vitamin A-fat loading test. The vitamin A-fat loading test specifically labels intestinally derived lipoproteins with retinyl palmitate (RP). The test was performed as recently described (15). After an overnight 12-h fast, subjects were given a meal containing 50 g fat/m^2 body surface (caloric content: 65% fat; 20% carbohydrate; 15%

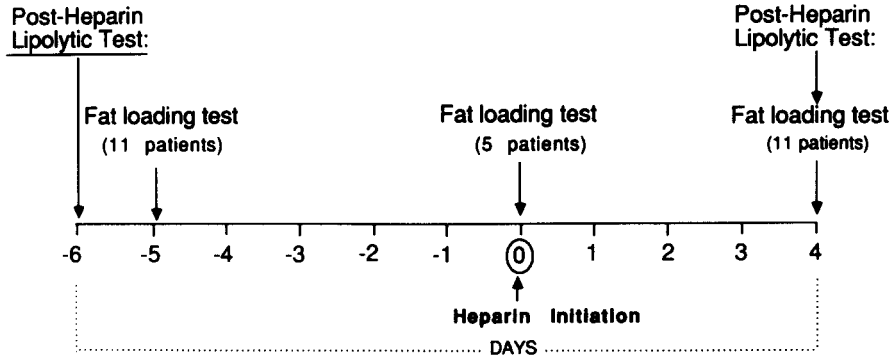

Fig. 1. A schematic description of study sequences. The study consisted of two phases: before and during heparin therapy. Fat loading tests and postheparin lipolytic activity tests were done in all of the patients in both study phases. In five patients an additional fat loading test was done on the first day of heparin administration.

protein) plus aqueous vitamin A, 60,000 U/m² body surface. The meal contained 600 mg cholesterol/1000 calories, and the P/S ratio was 0.3. It was provided in the form of a milkshake, scrambled eggs, bread, and cheese, which were eaten within 10 min. The vitamin A was added to the milkshake. After the meal, the subjects fasted for 10 h, with water provided ad libitum. To measure levels of RP, blood samples were drawn before the meal and every hour after the meal for 6 h, and then every 2 h until the tenth hour. The subjects tolerated the meal well, and none had diarrhea or other symptoms of malabsorption.

Analysis of samples. Venous blood was drawn from the forearm and transferred to a tube containing sodium EDTA. Samples were immediately centrifuged at 1500 *g* for 15 min; 1 ml plasma was stored in foil wrap at −20°C for retinyl ester assay, and another 0.5 ml was stored at 4°C for triglyceride determinations. An aliquot of 2.5 ml plasma was transferred to a 0.5 × 2 inch cellulose nitrate tube, overlaid with 2.5 ml sodium chloride solution (d 1.006 g/ml), and subjected to preparative ultracentrifugation for 1.6×10^6 *g*-min in an SW-55 rotor (Beckman Instruments, Inc., Fullerton, CA) to float chylomicron particles of $S_f > 1000$ (36–38).

The chylomicron-containing supernatant was removed and brought to a total volume of 2 ml with saline. The infranatant was brought to a volume of 5 ml with saline. Aliquots of supernatant and infranatant (0.5 ml each) were wrapped in foil and assayed for retinyl ester. Addi tional aliquots were assayed for triglyceride concentration. As discussed elsewhere, the procedure apparently separates a predominantly chylomicron population from a predominantly chylomicron remnant population (15, 38).

Retinyl ester assay. The assays were carried out in dim light with HPLC grade solvents. Retinyl acetate was added to the samples as an internal standard. The samples were then mixed with 4 ml ethanol, 5 ml hexane, and 4 ml water, with vortexing between each addition. Two phases were formed, and 4 ml of the upper (hexane) phase was removed and evaporated under nitrogen. The residue was dissolved in a small volume of benzene, and an aliquot was injected into an HPLC 5 μg ODS-18 radial compression column. Methanol (100%) was used as the mobile phase at a flow rate of 2 ml/min. The effluent was monitored at 340 nm, and peak RP was identified by comparison to the retention time of a purified standard (Sigma Chemical Co., St. Louis, MO) (39). In agreement with previous reports (40), it was found that 75–80% of total plasma retinyl esters were accounted for by RP. In addition, the distribution of retinyl esters remained constant throughout the study.

Lipid and lipoprotein determinations. Cholesterol and triglyceride levels were measured enzymatically using the reagents cholesterol 236691 and triglyceride 126012 (Boehringer Mannheim, Inc., Indianapolis, IN). HDL cholesterol was determined after precipitation of whole plasma with dextran sulfate–magnesium.

Determination of lipoprotein lipase and hepatic triglyceride hydrolase activities. Lipase activities were determined in plasma before and after the injection of intravenous heparin, 60 U/kg. Plasma was kept frozen at −20°C until performance of the assay. Lipase was assayed as described by Huttunen et al. (41). Lipase activities were measured using [³H]triolein emulsified in gum arabic as previously described (42). The test was performed twice: first, in whole plasma containing both LPL and HTGL, and second, in the presence of an antibody to HTGL. The antibody was supplied by Dr. T. Olivecrona (University of Umea, Sweden) and raised in rabbits against purified human HTGL. LPL activity was derived from the sample containing the antibody, and HTGL activity was calculated as the difference between whole plasma activity and activity measured in the presence of the antibody. The same method was used to determine the low lipase activities in pre-heparin plasma samples.

Statistical analysis

Paired Student's *t* test was used for statistical analysis; $P < 0.05$ was considered significant. The amounts of RP in total plasma, plasma chylomicron fraction, and plasma chylomicron remnant fraction were quantified by the area ratio method (43), using retinyl acetate as a reference (15).

RESULTS

The effect of 4 days of heparin therapy and/or hospitalization fasting lipids and lipoproteins is shown in **Table 1.** Neither heparin administration nor hospitalization per se had an effect on fasting plasma lipids or on lipoprotein levels.

The post-heparin lipase activity test showed that the lipolytic activity of the two key enzymes before therapy was significantly different from that 4 days after initiation of heparin (**Table 2**). Measurements were performed twice at each period: before the intravenous injection of bolus heparin 60 U/kg and 15 min after the injection. No or only minimal enzyme activities were observed before the injection of bolus heparin, when the assay was performed at the beginning of the study (prior to heparin therapy). However, after 4 days of continuous heparin administration, consistent, albeit low-level, activities were found for the two enzymes. The increase in LPL activity was significant ($P < 0.02$), and the increase in HTGL was borderline significant ($P < 0.071$). When lipase activities were measured 15 min after injection of bolus heparin, a very different pattern was observed. Both lipolytic activities on day 4 were significantly lower than those found before heparin therapy was begun. LPL levels now measured 1.85 ± 1.1 vs. 4.1 ± 1.3 μmole FFA/ml per h ($P < 0.0005$), and HTGL levels 1.3 ± 0.69 vs. 3.4 ± 1.7

TABLE 1. Effect of 4 days of heparin administration and/or hospitalization on fasting lipids and lipoproteins

Subject	Age	Sex	BSA	Total Cholesterol		Triglyceride		LDL-Cholesterol		HDL-Cholesterol	
				Pre-Hep	On Hep	Pre-Hep	On Hep	Pre-Hep	On Hep	Pre-Hep	On Hep
	yr		m^2	*mg/dl*							
Study group											
1	67	F	1.7	225	222	175	176	153	151	47	46
2	68	M	1.95	234	203	115	137	146	137	69	43
3	87	M	1.75	176	202	49	58	100	119	68	73
4	67	M	1.95	238	225	380	215	146	144	29	43
5	74	M	1.8	136	160	155	198	85	94	25	33
6	31	M	1.65	172	127	87	78	151	78	37	36
7	80	F	1.55	289	288	252	260	198	194	49	51
8	76	M	1.73	219	204	114	127	165	150	35	33
9	79	M	1.75	218	222	81	87	146	149	53	59
10	74	F	1.55	259	233	280	200	167	154	45	46
11	80	M	1.8	180	206	138	119	119	137	38	49
Mean	71		1.74	211	206	163	150	142	137	45	46
± SD	± 14		± 0.13	± 44	± 41	± 102	± 64	± 31	± 31	± 14	± 12
P value				NS		NS		NS		NS	
				Hospitalization Days							
				3–4	12–14	3–4	12–14	3–4	12–14	3–4	12–14
Control group											
1	64	F	1.81	206	197	185	194	125	118	44	40
2	80	M	1.76	204	198	144	168	126	118	49	46
3	68	M	1.92	215	204	175	172	144	131	36	38
4	75	M	1.93	210	196	97	110	136	125	55	49
5	71	M	1.84	168	173	134	126	92	103	49	45
6	65	F	1.69	244	226	236	214	156	146	38	37
Mean	70.5		1.82	207	199	161	164	129	123	45	42
± SD	± 6		± 0.09	± 23	± 16	± 47	± 39	± 21	± 14	± 7	± 4
P value				NS		NS		NS		NS	

BSA, body surface area; hep, heparin.

μmole FFA/ml per h ($P < 0.007$). In the control group, no differences in lipolytic activity were found between days 3–4 and 12–14 of hospitalization (Table 2).

The effect of 4 days of heparin treatment on plasma postprandial lipoprotein levels is shown in **Table 3** and **Fig. 2.** By the fourth day of treatment, peak plasma RP levels and area below the plasma RP curves increased significantly from pretreatment levels (2628 ± 1275 to 4336 ± 1987 μg/l, $P < 0.001$ and 15154 ± 5841 to 24392 ± 9871 μg/l·h, $P < 0.005$, respectively). This effect was caused by an increase in both the chylomicron and nonchylomicron fractions, but mainly by the first. On day 4, mean peak chylomicron RP levels increased from 1882 ± 669 to 3166 ± 1283 μg/l ($P < 0.007$), and the area from 11091 ± 4393 to 17684 ± 8949 μg/l·h ($P < 0.003$); mean peak nonchylomicron RP levels changed from 891 ± 371 to 1169 ± 485 μg/l ($P < 0.05$), and the area from 4118 ± 2492 to 6623 ± 790 μg/l·h ($P < 0.01$).

The postprandial increase in plasma triglyceride levels is shown in Table 3 and **Fig. 3.** Mean peak triglyceride level was 237 ± 176 mg/dl before treatment and 314 ± 249 mg/dl on day 4 of heparin therapy ($P < 0.005$); the area below the curves was 1568 ± 988 and 2046 ± 1242 mg/dl·h, respectively ($P < 0.002$). The individual responses of each of the patients to heparin treatment as measured by the areas below the RP and triglyceride curves are shown in **Fig. 4.** Apparently, heparin had the same effect on postprandial lipoprotein metabolism in all of the study patients.

Hospitalization per se had no effect on postprandial RP and triglyceride levels as measured in six control subjects on days 3–4 and 12–14 of hospitalization (Table 2).

To study the effect of acute heparin administration, an additional fat loading test was done in five of the patients on the first day of heparin treatment. At onset of heparin administration (2 h after ingestion of the fatty meal), chylomicron RP immediately stopped rising and then decreased to levels significantly lower than those measured in the test done before heparin therapy. It remained at this low level for the duration of the test (**Fig. 5**). Mean

TABLE 2. Effect of heparin therapy on lipolytic activity

	Pre-Heparin Therapy				On Heparin Therapy			
	Time 0		15 min after Heparin Bolus		Time 0		15 min after Heparin Bolus	
Subject	LPL	HTGL	LPL	HTGL	LPL	HTGL	LPL	HTGL
Study group								
1	0.02	0.027	2.3	1.4	0.6	0.6	0.7	1.0
2	0.21	0.09	5.3	2.3	–	–	0.6	1.0
3	0.96	0.08	3.3	7.3	–	–	1.3	0.7
4	0.14	0.01	3.6	2.1	1.3	0.1	2.3	0.6
5	0.03	0	2.6	2.8	0.9	1.1	1.8	1.5
6	0.19	0	4.6	3.3	1.6	0.4	4.0	1.3
7	0.21	0	3.8	5.2	–	–	0.8	0.9
8	0	0	5.6	2.9	1.2	0.2	1.8	1.1
9	1.5	0.84	3.2	4.2	–	–	2.0	2.0
10	1.51	0	6.6	2.2	3.1	1.7	3.2	2.9
Mean	0.4	0.10	4.1	3.4	1.3	0.68	1.85	1.3
± SD	± 0.09	± 0.2	± 1.3	± 1.7	± 0.9	± 0.6	± 1.10	± 0.69
P value					0.017	0.071	0.0005	0.007
	Hospitalization, Days 3-4				Hospitalization, Days 12-14			
Control group								
1	0.21	0.032	4.8	2.2	0.14	0.03	6.2	3.5
2	0.12	0.07	4.6	3.2	0.32	0.02	4.1	4.0
3	1.3	0.45	3.6	5.2	0.68	0.36	3.0	4.8
4	0.36	0.12	6.8	4.2	0.21	0.08	7.2	3.6
5	0.52	0	3.3	2.1	0.25	0.06	4.6	3.2
6	0.67	0.08	5.4	3.5	0.54	0.11	6.2	3.8
Mean	0.53	0.12	4.75	3.4	0.35	0.11	5.21	3.81
± SD	± 0.42	± 0.16	± 1.2	± 1.1	± 0.2	± 0.1	± 1.5	± 0.5
P value			NS					

TABLE 3. Effects of 4 days of heparin administration on postprandial retinyl palmitate (RP) and triglyceride (TG) levels

	Peak Levels				Areas under Curves			
Group	TG	Plasma RP	Chylo RP	Non-Chylo RP	TG	Plasma RP	Chylo RP	Non-Chylo RP
	mg/dl		*μg/l*		*mg/dl · h*		*mg/dl · h*	
Study group								
Before heparin	237	2628	1882	891	1568	15154	11091	4118
(n = 11)	± 176	± 1275	± 669	± 317	± 982	± 5841	± 4393	± 2492
On heparin	314	4336	3166	1169	2046	24392	17684	6623
(n = 11)	± 249	± 1987	± 1283	± 385	± 1242	± 9871	± 8949	± 1790
P value	0.005	0.001	0.007	0.05	0.002	0.0005	0.003	0.01
Control group								
Hosp. Days 3-4	216	2496	1754	812	1342	13766	9846	3966
(n = 6)	± 89	± 742	± 544	± 184	± 580	± 3280	± 2100	± 1360
Hosp. Days 12-14	246	2645	1851	743	1434	14843	10245	4463
(n = 6)	± 124	± 815	± 560	± 242	± 601	± 2800	± 3643	± 1840
P value	NS	NS	NS	NS	NS	NS	NS	NS

Chylo, chylomicrons; Hosp, hospitalization. Values given as mean ± SD.

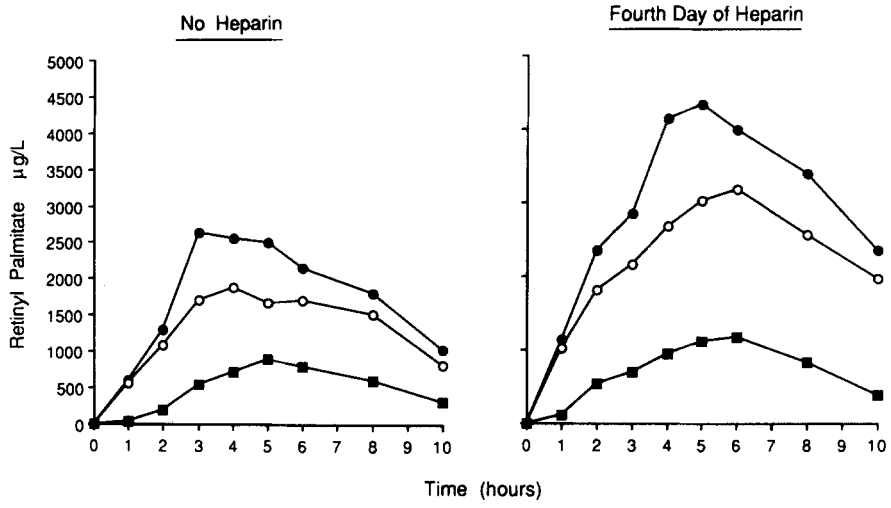

Fig. 2. Effects of 4 days of heparin administration on postprandial lipoprotein levels. Total plasma (—●—), chylomicron (—○—), and chylomicron remnant (—■—) retinyl palmitate responses before (left) and on the fourth day (right) of heparin therapy.

peak chylomicron RP level before heparin treatment was 2907 ± 919 µg/l. On the first day of heparin, chylomicron RP was 2470 ± 870 µg/ml ($P < 0.05$). Mean RP areas measured 16678 ± 6895 µg/l · h and 10474 ± 3893 µg/l · h, respectively ($P < 0.05$). Chylomicron remnant RP levels increased sharply 1 h after the beginning of heparin therapy and then decreased to levels lower than in the pretreatment period. However, peak chylomicron remnant RP levels and areas below the curves before and after heparin treatment were not significantly different. The acute onset of heparin also caused a significant decrease in postprandial TG levels. The areas below the plasma TG curves were 1578 vs. 1088 mg/dl · h ($P < 0.05$) (**Table 4, Fig. 6**).

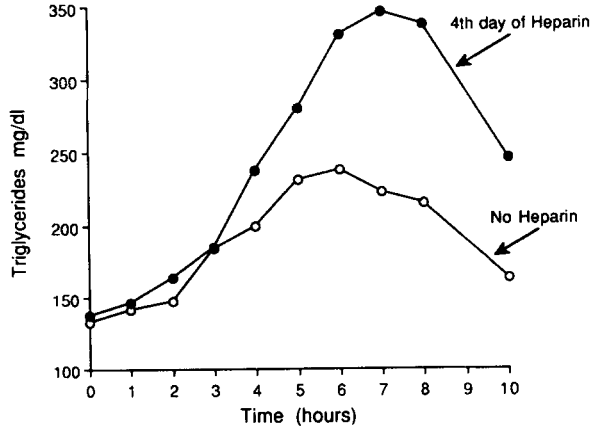

Fig. 3. Effects of 4 days of heparin therapy on postprandial plasma triglyceride levels.

DISCUSSION

This study clearly demonstrates that 4 days of continuous intravenous administration of heparin causes a severe disturbance in postprandial lipoprotein metabolism, with accumulation of these lipoproteins in the circulation. Fasting lipids and lipoproteins are not affected.

Postprandial lipoprotein metabolism is considered to occur in two stages. Initially, the chylomicrons interact with LPL in the extrahepatic tissues, resulting in triglyceride hydrolysis and delivery of the free fatty acids to the tissues. After most of the triglycerides are hydrolyzed, chylomicron remnant particles are formed (44, 45). These are rapidly removed from the circulation by hepatocyte receptors that recognize apoE (46). The vitamin A-fat loading test used in the present study has an advantage in that it specifically follows both chylomicron and chylomicron remnant metabolism (15, 40) and is able to detect disturbances, if they exist, in each of the metabolic stages.

Our results showed accumulation of chylomicrons in the plasma on the fourth day of heparin therapy. This indicates that prolonged heparin administration causes disturbances in the first (lipolytic) stage of chylomicron metabolism. Indeed, we found a marked decrease in the activity of the two key enzymes, LPL and HTGL, that may affect the process. To confirm that these changes were secondary to heparin administration and not to the conditions of hospitalization per se, the same tests were performed on six control patients hospitalized for mild brain ischemic events under similar conditions but not placed on heparin therapy. No changes in postprandial lipoprotein metabolism or in lipolytic activity were found in these patients during hospitalization. We suggest that the deple-

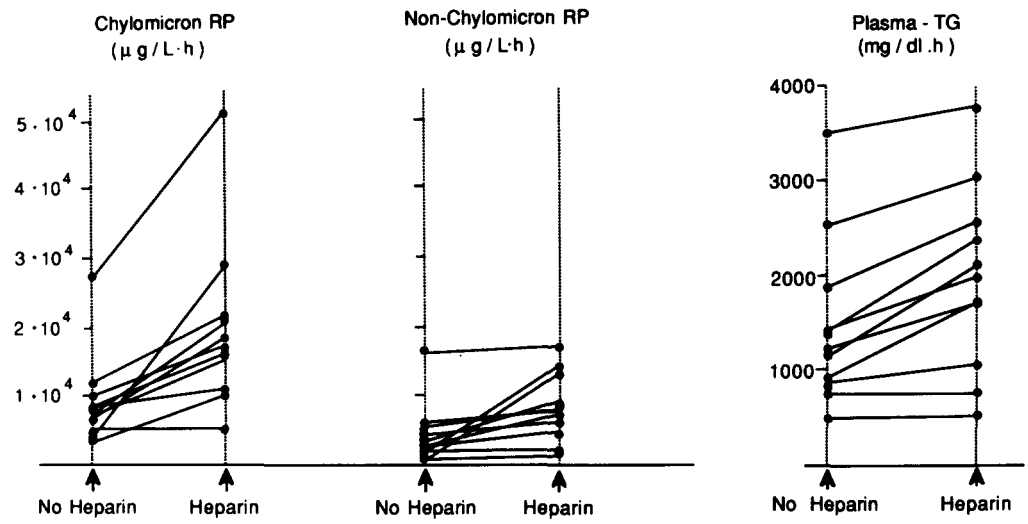

Fig. 4. Individual response to 4 days of heparin administration. The chylomicron (left), chylomicron remnant (mid) retinyl palmitate areas, and areas below the plasma triglyceride curves (right) for each of the 11 patients are shown before and during heparin therapy.

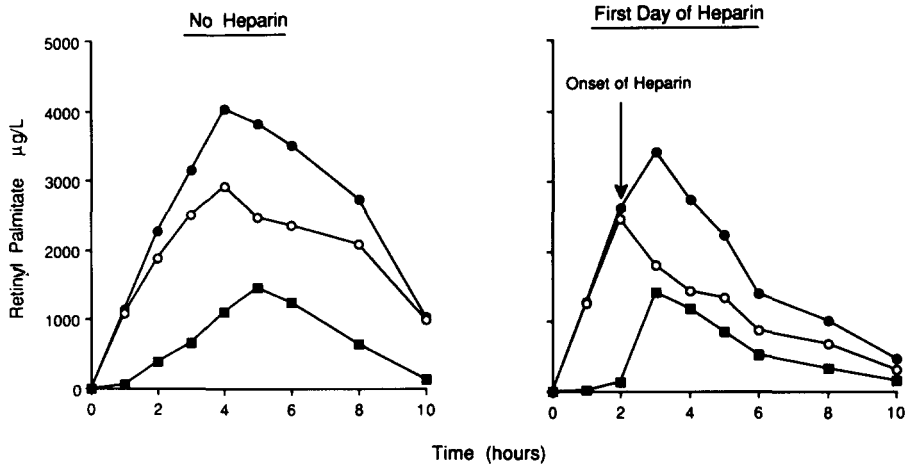

Fig. 5. Effect of acute heparin administration on postprandial lipoprotein levels. Total plasma (—●—), chylomicron (—○—), and chylomicron remnant (—■—) retinyl palmitate responses are shown before heparin therapy (left) and on first day of heparin therapy (right).

TABLE 4. Effects of acute heparin administration on postprandial retinyl palmitate (RP) and triglyceride (TG) levels

Study Period	Peak Levels				Areas under Curves			
	TG	Plasma RP	Chylo RP	Non-Chylo RP	TG	Plasma RP	Chylo RP	Non-Chylo RP
	mg/dl		*μg/l*		*mg/dl · h*		*mg/dl · h*	
Before heparin (n = 5)	237 ± 177	4018 ± 1672	2907 ± 919	1447 ± 815	1578 ± 1037	22587 ± 9394	16678 ± 6895	5909 ± 2293
On 1st day of heparin (n = 5)	172 ± 106	3417 ± 1423	2470 ± 870	1407 ± 652	1088 ± 610	15404 ± 4817	10474 ± 3893	4210 ± 1594
P value	0.072	0.142	0.042	0.723	0.034	0.028	0.021	0.196

Values given as mean ± SD.

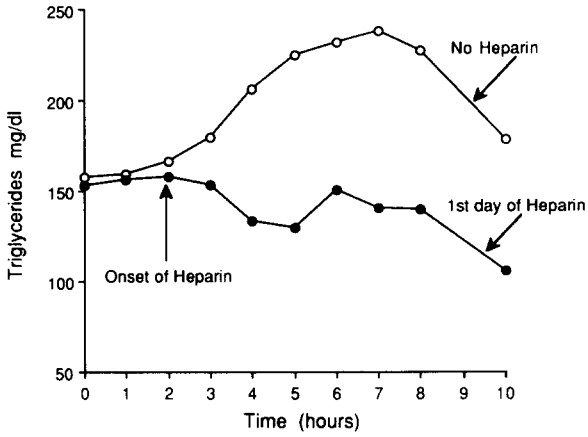

Fig. 6. The effect of acute heparin administration on plasma postprandial triglyceride levels.

tion of the two enzymes found in the study patients was due to their continuous release from endothelial sites during the 4 days of heparin administration. The level of endothelial LPL activity is dependent on its production in nearby parenchymal cells (47), on its blood transport to and along endothelial wall from one binding site to another, and on its avid uptake in liver. This system is not in equilibrium; there is a concentration gradient from the area with LPL synthesis to other parts of the vascular bed, with a net flow of LPL to the liver (48). Thus, a constant increased release of LPL from its sites by a continuous heparin drip may exhaust the tissue capacity to synthesize and/or transport the enzymes to the endothelium. The lack of an effect on fasting triglyceride may reflect the capacity of the enzymatic system to cope with the smaller mass of endogenous triglycerides as compared to the much larger load of dietary lipids. Alternatively, continuous low-grade hydrolysis of triglycerides in the plasma may have caused extraction of fatty acids by the liver and enhanced VLDL secretion. After 4 days, the levels of the nonchylomicron fraction also increased significantly, but to a lesser extent than the chylomicron fraction.

Does heparin treatment affect chylomicron remnant metabolism? We do not know. Our nonchylomicron fraction consisted predominantly of chylomicron remnants, but it also contained a certain amount of small chylomicrons of $S_f < 1000$ (15). It is possible, therefore, that the increase in this fraction after heparin administration reflects the inhibition of lipolysis of these small chylomicrons, similar to the large chylomicrons. A direct effect of continuous heparin administration on remnant clearance, however, cannot be ruled out.

Berr, Eckel, and Kern (34) showed that acute heparin administration enhanced hydrolysis of chylomicron triglycerides and formation of chylomicron remnants but had no effect on the metabolism of chylomicron remnants. We examined the effect of acute heparin administration in five patients. We found, in agreement with others (49, 50), that chylomicron fractions significantly decreased, suggesting a more rapid lipolytic rate. The nonchylomicron fraction also decreased, but this result was not statistically significant. Thus, acute heparin administration enhances chylomicron lipolysis, while 4 days of continuous intravenous administration inhibits chylomicron lipolysis. Neither acute nor, most probably, prolonged administration affects the rate of chylomicron remnant disappearance.

Most of our lives are spent in the postprandial state, during which time the vessel walls are exposed to postprandial lipoproteins. Experimental, clinical, and in vitro studies have indicated that postprandial lipoproteins may be particularly atherogenic (28–30). These particles are metabolized on the endothelial surface of arteries, where their cholesterol may be incorporated into the vessel wall and may stimulate atherosclerotic lesion formation. Our study demonstrates that 4 days of heparin administration increases levels of chylomicrons in the circulation, thus exposing vessel walls to more of these particles for an extended period of time. This should be taken into consideration before such treatment is recommended. However, our study is limited to 4 days of heparin treatment only and was done mainly on an elderly population with thromboembolic disease. More studies on longer periods of heparin therapy and on younger patients should be performed to examine whether the changes demonstrated in our study are persistent or become worse, or whether some adaptation occurs over time.

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REFERENCES

1. Buchwald, H., T. D. Rohde, P. D. Schneider, R. L. Varco, and P. J. Blackshear. 1980. Long term, continuous intravenous heparin administration by an implantable infusion pump in ambulatory patients with recurrent venous thrombosis. *Surgery.* **88:** 507–516.
2. Engelberg, H. 1984. Heparin and the atherosclerotic process. *Pharmacol. Rev.* **36:** 91–110.
3. Neri Serneri, G. G., F. Rovelli, G. F. Ganaini, S. Pirelli, M. Carrovali, and A. Fortini. 1987. Effectiveness of low-dose heparin in prevention of myocardial reinfarction. *Lancet.* **25:** 937–942.
4. Neri Serneri, G. G., R. Abate, and D. Prisco. 1988. Decrease in frequency of anginal episodes by control of thrombin generation with low-dose heparin: a controlled crossover randomized study. *Am. Heart J.* **115:** 60–67.
5. Hahn, P. F. 1943. Abolishment of alimentary lipemia following injection of heparin. *Science.* **98:** 19–20.
6. Korn, E. D. 1955. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. II. Substrate specificity and

activation of coconut oil. *J. Biol. Chem.* **215:** 1-26.
7. Robinson, D. S., and P. M. Harris. 1959. The production of lipolytic activity in the circulation of the hind limb in response to heparin. *Q. J. Exp. Physiol.* **44:** 80-90.
8. Spitzer, J. A., and J. J. Spitzer. 1956. Effect of liver on lipolysis by normal and postheparin sera in the rat. *Am. J. Physiol.* **185:** 18-22.
9. La Rosa, J. C., R. I. Levy, H. G. Windmueller, and D. S. Fredrickson. 1972. Comparison of the triglyceride lipase of liver, adipose tissue and postheparin plasma. *J. Lipid Res.* **13:** 356-363.
10. Nilssen-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49:** 667-693.
11. Iverius, P-H., and A-M. Östlund-Lindqvist. 1976. Lipoprotein lipase from bovine milk. Isolation procedure, chemical characterization and molecular weight analysis. *J. Biol. Chem.* **251:** 7791-7795.
12. Nilssen-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1976. Intra- and extracellular forms of lipoprotein lipase in adipose tissue. *Biochim. Biophys. Acta.* **431:** 147-156.
13. Olivecrona, T., G. Bengtsson, S-E. Marklund, U. Lindahl, and M. Hook. 1977. Heparin-lipoprotein lipase interactions. *Fed. Proc.* **36:** 60-65.
14. Nikkila, E. A., T. Kuusi, M-R. Taskinen, and M. J. Tikkanen. 1984. Regulation of lipoprotein metabolism by endothelial lipolytic enzymes. *In* Treatment of Hyperlipoproteinemia. L. A. Carlson and A. G. Olsson, editors. Raven Press, New York. 1-34.
15. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III and type IV hyperlipoproteinemic individuals. Effects of treatment with cholestyramine and gemfibrozil. *J. Clin. Invest.* **79:** 1110-1119.
16. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism in vivo and in vitro studies in man. *Eur. J. Clin. Invest.* **10:** 487-495.
17. Jansen, H., A. van Tol, and W. C. Hülsmann. 1980. On the metabolic function of heparin releasable liver lipase. *Biochem. Biophys. Res. Commun.* **92:** 53-59.
18. Murase, T., and H. Itakura. 1981. Accumulation of intermediate density lipoprotein in plasma after intravenous administration of hepatic triglyceride lipase antibody in rats. *Atherosclerosis.* **39:** 293-300.
19. Breckenridge, W. C., J. A. Little, and P. Alaupovic. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161-179.
20. Goldberg, I. J., N. Anhle, J. R. Paterniti, H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184-1192.
21. Daggy, B. P., and A. Bensadoun. 1986. Enrichment of apolipoprotein B-48 in the LDL density class following in vivo inhibition of hepatic lipase. *Biochim. Biophys. Acta.* **877:** 259-261.
22. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1968. Hypertriglyceridemia: a metabolic consequence of chronic renal failure. *N. Engl. J. Med.* **279:** 181-185.
23. Ibels, L. S., M. F. Reardon, and P. J. Nestel. 1976. Plasma post-heparin lipolytic activity and triglyceride clearance in uremic and hemodialysis patients and renal allograft recipients. *J. Lab. Clin. Med.* **87:** 648-658.
24. Chan, M. K., Z. Varghese, J. W. Persaud, R. A. Baillod, and J. F. Moorhead. 1980. Fat clearance before and after heparin in chronic renal failure—haemodialysis reduces post-heparin fractional clearance rates of intralipid. *Clin. Chim. Acta.* **108:** 95-101.
25. Persson, E., J. Nordenstrom, and E. Vinnars. 1987. Plasma clearance of fat emulsion during continuous heparin infusion. *Acta Anaesthesiol. Scand.* **31:** 189-192.
26. Lindholm, M., and S. Rossner. 1982. Rate of elimination of the intralipid fat emulsion from circulation in ICU patients. *Crit. Care Med.* **10:** 740-746.
27. Goldberg, D. M., and T. Chajek-Shaul. 1990. Effect of chronic heparin administration on serum lipolytic activity and some aspects of lipid metabolism. *Biochim. Biophys. Acta.* **1047:** 103-111.
28. Zilversmit, D. B. 1979. Atherosclerosis—a postprandial phenomenon. *Circulation.* **60:** 473-485.
29. Zilversmit, D. B. 1984. Postprandial hyperlipidemia and its relation to atherosclerosis. *In* Latent Dyslipoproteinemias and Atherosclerosis. J. L. de Gennes, editor. Raven Press, New York. 1-8.
30. Mahley, R. W. 1979. Dietary fat, cholesterol, and accelerated atherosclerosis. *In* Atherosclerosis Review. R. Paoletti and A. M. Gotto, editors. Raven Press, New York. 1-34.
31. Hazzard, W. R., and E. L. Bierman. 1976. Delayed clearance of chylomicron remnants following vitamin A containing oral fat load in broad-B disease (type III hyperlipoproteinemia). *Metab. Clin. Exp.* **25:** 777-801.
32. Wilson, D. E., I. Chan, K. N. Buch, and S. C. Herton. 1985. Postchallenge plasma lipoprotein retinoids: chylomicron remnants in endogenous hypertriglyceridemia. *Metab. Clin. Exp.* **34:** 551-558.
33. Cortner, J. A., P. M. Coates, N-A. Le, D. R. Cryer, M. C. Ragni, A. Faulkner, and T. Langer. 1987. Kinetics of chylomicron remnant clearance in normal and in hyperlipoproteinemic subjects. *J. Lipid Res.* **28:** 195-206.
34. Berr, F., R. H. Eckel, and F. Kern. 1985. Plasma decay of chylomicron remnants is not affected by heparin-stimulated plasma lipolytic activity in normal fasting man. *J. Lipid Res.* **26:** 852-859.
35. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J. Clin. Invest.* **80:** 1471-1477.
36. Grundy, S. M., and H. Y. I. Mok. 1976. Chylomicron clearance in normal and hyperlipidemic man. *Metab. Clin. Exp.* **25:** 1225-1239.
37. Dale, V. P., and J. I. Hamlin. 1962. Particulate fat in lymph and blood. *Physiol. Rev.* **42:** 674-701.
38. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. S. Nelson, editor. Wiley Interscience, New York. 181-274.
39. Blomhoff, R., M. Rasmussen, A. Nilsson, K. R. Norum, T. Berg, W. S. Blaner, M. Kato, J. R. Mertz, D. S. Goodman, U. Eriksson, and P. A. Peterson. 1985. Hepatic retinoid metabolism: distribution of retinoids, enzymes and binding proteins in isolated rat liver cells. *J. Biol. Chem.* **260:** 13560-13565.
40. Berr, F., and F. Kern, Jr. 1984. Plasma clearance of chylomicrons labeled with retinyl palmitate in healthy human subjects. *J. Lipid Res.* **25:** 805-812.
41. Huttunen, J. K., C. Ehnholm, M. Kekki, and E. A. Nikkila. 1976. Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with

hypertriglyceridaemia: correlation to sex, age and various parameters of triglyceride metabolism. *Clin. Sci. Mol. Med.* **50:** 249–260.
42. Eisenberg, S., D. Gavish, Y. Oschry, M. Fainaru, and R. J. Deckelbaum. 1984. Abnormalities in very low, low, and high density lipoproteins in hypertriglyceridemia. *J. Clin. Invest.* **74:** 470–482.
43. De Ruyten, M. G. M., and A. P. Dellenheer. 1978. Simultaneous determination of retinol and retinyl esters in serum or plasma by reversed phase high performance liquid chromatography. *Clin. Chem.* **24:** 1920–1923.
44. Blanchette-Mackie, E. J., and R. O. Scow. 1971. Sites of lipoprotein lipase activity in adipose tissue perfused with chylomicrons. Electron microscopic, cytochemical study. *J. Cell Biol.* **51:** 1–25.
45. Schoefe, G. I., and J. E. French. 1968. Vascular permeability to particulate fat: morphological observation on levels of lactating mammary gland and of lung. *Proc. R. Soc. (Biol).* **169:** 153–165.
46. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes: metabolically distinct apoE and apoB,E receptors. *J. Biol. Chem.* **256:** 5646–5655.
47. Eneback, S., H. Somb, J. Tavernier, G. Bjürsell, and T. Olivecrona. 1988. Tissue-specific regulation of guinea pig lipoprotein lipase: effects of nutritional state and of tumor necrosis factor on mRNA levels in adipose tissue, heart and liver. *Gene.* **64:** 97–106.
48. Olivecrona, T., and G. Bengtsson-Olivecrona. 1989. Heparin and lipases. *In* Heparin. D. Lane and U. Lindahl, editors. Edward Arnold, London. 335–361.
49. Dhanireddy, R., M. Hamosh, K. N. Swasubramaniah, P. Chowdhry, J. W. Scanlon, and P. Hamosh. 1981. Postheparin lipolytic activity and intralipid clearance in very-low-birth-weight infants. *J. Pediatr.* **98:** 617–622.
50. Benderly, A., E. Rosenthal, J. Levi, and G. Brook. 1983. Effect of heparin on lipoprotein profile during parenteral fat infusions. *J. Parenter. Enteral. Nutr.* **7:** 37–39.