Plasma squalene: lipoprotein distribution and kinetic analysis[1]

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Abstract Plasma squalene concentration is increased in hypertriglyceridemia. In 24 normotriglyceridemic and 12 hypertriglyceridemic subjects, whole plasma squalene correlated strongly with plasma triglyceride ($r = 0.973$, $P < 0.001$) in the latter. In normal postabsorptive plasma, squalene was found in each lipoprotein fraction, 50.8% in very low density lipoprotein, 25.6% in low density lipoprotein, and 23.6% in high density lipoprotein. When plasma triglyceride was increased by dietary intake in humans or by experimental diabetes in rats, plasma squalene increased correspondingly. Conversion of [^{14}C]mevalonic acid into [^{14}C]squalene and kinetic analysis of [^{14}C]squalene die-away curves were studied in 17 subjects. Hypertriglyceridemia significantly increased the estimated metabolically active plasma squalene pool. This together with an increase in radioactivity of squalene (dpm/ml plasma) in hypertriglyceridemia suggested that squalene production was increased. Squalene specific activity curves in lipoprotein fractions from four chylomicronemic subjects demonstrated that each fraction had newly synthesized squalene and that total plasma squalene kinetics represent the composite of several individual die-away curves. We conclude that squalene in whole plasma and in lipoprotein fractions varies directly with triglyceride content. Hypertriglyceridemia expands the plasma pool of metabolically active squalene, and each lipoprotein fraction contains squalene that is metabolically active in cholesterol synthesis.

Supplementary key words hypertriglyceridemia · VLDL · LDL · HDL

Liu and colleagues (1) have used the kinetics of isotopically labeled squalene in plasma to estimate cholesterol synthesis in man. Rapid equilibration between cellular and plasma squalene is a necessary assumption if this kinetic analysis is to reflect intracellular cholesterol synthesis. The assumption has been supported in man by the appearance of [^{14}C]-squalene in plasma within 2 min of [^{14}C]mevalonic acid administration (1); it has also been supported by the empirical agreement of data derived from squalene kinetic analysis with those derived from cholesterol balance techniques (1, 2). Abnormally high plasma triglyceride concentration, however, may change the normal dynamics of plasma squalene.

Goodman (3) showed in 1964 that triglyceride-rich lipoprotein (d < 1.019 g/ml) contains most newly synthesized squalene. The observation was made in a human subject by measuring the [^{14}C]squalene distribution in lipoprotein fractions after intravenous administration of ^{14}C-MVA. Liu et al. (4) found an elevated plasma squalene concentration in hypertriglyceridemic (HTG) subjects. There has been no published analysis of the distribution of squalene in plasma lipoprotein fractions, nor has the relationship been studied between plasma squalene and endogenous or exogenous triglyceride or the kinetics of plasma squalene in HTG. Each of these questions is significant in understanding the physiology of active plasma squalene pools and the factors affecting squalene kinetics in HTG.

The present study confirms the strong correlations between plasma triglyceride and plasma squalene concentrations in the HTG human and rat. It describes the distribution of plasma squalene in lipoprotein fractions of normal and HTG humans, and the effect of dietary triglyceride on plasma squalene. Finally, it describes the appearance and turnover of newly synthesized [^{14}C]squalene in lipoprotein fractions of HTG plasma.

METHODS

Thirty-six subjects were studied; pertinent clinical features are listed in **Table 1.** Subjects No. 1–9 were normal controls, with no evidence of disease. Subjects No. 10–24 were normotriglyceridemic (NTG), de-

Abbreviations: ^{14}C-MVA, DL[5-^{14}C]mevalonic acid; NTG, normotriglyceridemic; HTG, hypertriglyceridemia; VLDL, very low density lipoprotein (d < 1.006 g/ml); LDL, low density lipoproteins (d 1.006–1.063 g/ml); HDL, high density lipoproteins (d > 1.063 g/ml); SCP, sterol and squalene carrier protein: TG, triglyceride.

[1] A portion of this work was reported in 1977 in *Clin. Res.* **25:** A499 (Abstr.), and was presented at the National Meeting, American Heart Association, Miami, Florida, November 1977.

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TABLE 1. Clinical features and post-absorptive plasma triglyceride, squalene, and cholesterol concentrations of human subjects

Subject	Sex	Age	Triglyceride	Squalene	Cholesterol	Diagnosis[a]	Treatment[b]
			mg/dl	*μg/dl*	*mg/dl*		
Normal							
1	M	35	47	16.4	142	N	
2	M	29	80	15.0	124	N	
3	F	23	63	12.3	142	N	
4	M	24	91	31.9	151	N	
5	M	29	119	18.2	175	N	
6	F	37	100	18.9	164	N	
7	F	25	123	15.9	220	N	
8	F	24	112	21.6	217	N	
9	M	26	136	22.6	162	N	
Mean		28	96.8	19.2	166.3		
SE		1.7	9.8	1.9	11.0		
Normotriglyceridemic							
10	F	39	94	34.0	333	HCh	
11	F	56	86	15.0	197	CTX	
12	M	51	106	23.0	342	HCh	Diet
13	M	71	43	10.1	186	DM;ASHD;COPD	Dig;Fur
14	F	45	108	19.5	210	DM;HT	Ins
15	M	67	116	29.5	232	DM;DJD	Ins
16	F	53	83	21.0	158	DM;DJD	Ins
17	F	84	140	12.3	176	DM;HT	Ins
18	F	65	121	33.8	300	HCh;ASHD;HT	Diet
19	M	62	120	8.0	204	DM;ASHD;PVD	Ins
20	F	64	78	19.4	260	DM;ASHD;HT	Ins
21	M	51	115	8.3	205	DM;HT	Ins
22	F	42	88	25.2	145	DM	Diet
23	F	65	46	14.5	226	DM	Ins
24	F	46	100	18.3	210	DM	Diet
Mean		57.4	96.2	19.5	225.6		
SE		3.2	6.9	2.2	15.3		
HTG without chylomicronemia							
25	F	59	229	31.3	448	DM;ASHD;HCh	Dig;Ins
26	M	76	234	35.0	306	HCh;AS	Pers;ASA
27	M	72	212	17.0	227	DM;AS	Ins
28	F	59	247	15.0	397	DM;AS;HCh	Ins
29	M	74	452	121.0	154	DM;ASHD;PVD	Dig;Fur
30	F	50	580	103.6	168	ASHD;DM	
31	F	55	202	26.3	254	DM	
Mean		63.6	308.0	49.9	279.1		
SE		3.9	55.8	16.4	42.1		
HTG with chylomicronemia							
32	M	43	5762	988	399	HTG;DM;ASHD	Ins
33	F	28	1110	122	171	HTG;ASHD	Diet
34	F	70	4676	552	541	HTG;DM;ASHD	Ins;Dig
35	F	66	1616	224	436	HTG;HT;DM	Dig
36	F	59	1072	228	296	HTG;DM	Diet
Mean		53	2847	423	369		
SE		7.9	988	159	63		

[a] Diagnostic abbreviations: N, normal; HCh, hypercholesterolemia (>300 mg/dl); CTX, cerebrotendinous xanthomatosis; DM, diabetes mellitus; ASHD, arteriosclerotic heart disease; COPD, chronic obstructive pulmonary disease; HT, hypertension; DJD, degenerative joint disease; PVD, peripheral vascular disease.

[b] Treatment abbreviations: Dig, digoxin; Fur, furosemide; Ins, insulin; Pers, persantin; ASA, acetylsalicylic acid.

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fined as postabsorptive triglyceride concentration <150 mg/dl with the disease states described. Subjects No. 25–31 were HTG (triglyceride concentration >200 mg/dl) without fasting chylomicronemia, while subjects No. 32–36 had fasting chylomicronemia (determined by lipoprotein electrophoresis and by observation of the plasma). All studies were approved by the Institutional Human Rights in Research Committee, and isotope studies were approved by the Radioisotope Usage Committee. All patients gave informed consent for their participation.

The normal control subjects took part only in the study of plasma squalene distribution in normal plasma (i.e., they were not administered an isotope). Squalene distribution in lipoprotein fractions of the other NTG group and of the nonchylomicronemic HTG group was studied; in 19 of these subjects a full squalene kinetic analysis was performed, allowing calculation of the metabolically active squalene pool size. Four of the five chylomicronemic subjects had analysis of squalene kinetics in each lipoprotein fraction; only with very high plasma triglyceride concentration was there sufficient plasma squalene to permit calculation of specific activity curves in each lipoprotein fraction.

Postabsorptive blood was drawn 12–16 hr after eating. The effect of diet was measured in 18 subjects admitted to the Cornell Clinical Research Center for 7–24 hr. Two standards were given, consisting of 500 calories each, distributed as 45% fat, 40% carbohydrate, and 15% protein. Squalene content of ingested food was estimated at less than 5 mg, based on published data (4).

The temporal appearance and lipoprotein distribution of newly synthesized squalene as well as the metabolically active pool size were determined after intravenous administration of 250 μCi of DL[5-^{14}C]MVA (Schwarz/Mann, Orangeburg, NY) with sp act of 17 mCi/mmol. Thirty μCi of [1,2-^{3}H]cholesterol (40 mCi/mmol) (New England Nuclear Corp., Boston, MA) was administered simultaneously to permit calculation of the fractional conversion of MVA to squalene (1). Isotope was mixed in 150 ml of 0.9% saline and infused with two saline washes. Residual isotope was extracted from all glassware and tubing. Blood samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hr after isotope infusion through an indwelling intravenous cannula in the arm contralateral to the infusion site. Twenty milliliters of blood were used when lipoprotein fractionation was performed; most samples required 10 ml of blood for squalene specific activity. The total volume of blood was 100–200 ml. Blood was anticoagulated with EDTA (1.4 mg/ml), and the chylomicron fraction was separated within 2 hr of sampling.

TABLE 2. Diabetic and control rats: plasma glucose, triglyceride, and squalene concentrations

	(*n*)	Glucose	Triglyceride	Squalene
		mg/dl	*mg/dl*	*μg/dl*
Control	(15)	92 ± 4.0[a]	224 ± 9.3	51 ± 6.3
Diabetic	(15)	482 ± 24.4	937 ± 108.8	351 ± 52.9

[a] Mean ± SEM.

Fifteen rats were made acutely diabetic by intravenous injection of streptozotocin, 65 mg/kg (kindly provided by Dr. W. E. Dulin, Upjohn Co., Kalamazoo, MI). Ether-anesthetized rats were exsanguinated, and plasma was taken for analysis 48 hr after streptozotocin administration. At this time the rats were hyperglycemic and polyuric but not acidotic. All rats with acute diabetes were hypertriglyceridemic (**Table 2**). Fifteen age-matched control rats were handled and fed identically, but were injected with streptozotocin-free buffer.

Data Analysis

Metabolically active plasma squalene pool size was estimated by computerized input–output analysis (1, 5) utilizing the formula

$$\text{Active Pool} = T\tfrac{1}{2}\,max \times I_T/0.693$$

where I_T is the input rate of squalene, calculated by the dose of administered squalene divided by the area under the specific activity curve, and $T\frac{1}{2}\,max$ is the maximum half-life of the decay exponential, calculated by least squares fit (6). Correlation coefficients were calculated with the least squares method. Comparison of means utilized the Student's *t* test (6) for unpaired data and the paired data in the case of a given patient's blood being sampled throughout the day.

Analytical procedures

Ultracentrifugation was performed on a Beckman L-550 ultracentrifuge with a swinging bucket rotor, by the method of Havel, Eder, and Bragdon (7). Chylomicrons were separated by layering plasma with saline (d 1.006 g/ml) and centrifuging for 30 min at 15,000 *g* at 20°C. The infranate was subjected to a second "chylomicron wash" using the same procedure and the supernates were collected. This technique slightly increases the apparent chylomicron concentration at the expense of VLDL (8). VLDL was separated from LDL + HDL by layering chylomicron-free plasma with saline of d 1.006 g/ml and centrifuging for 18 hr

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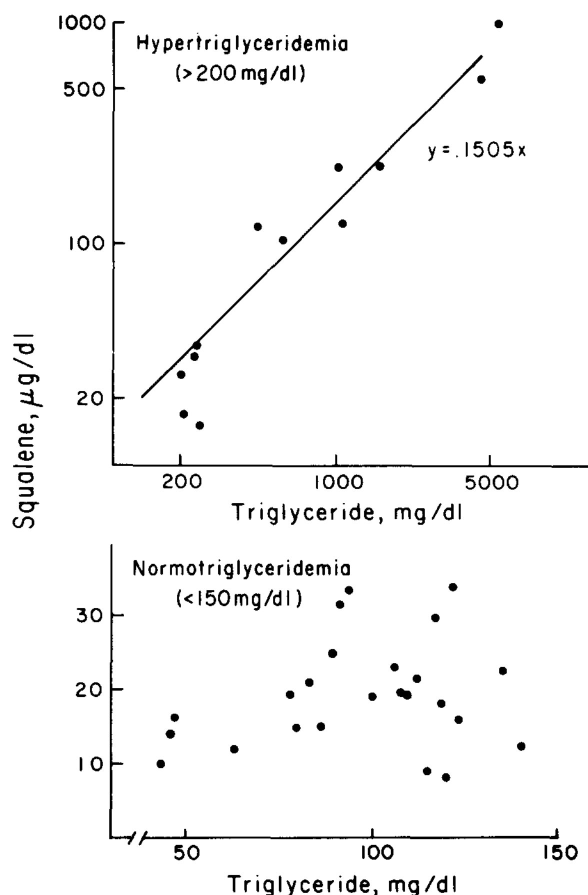

Fig. 1. Squalene vs. triglyceride in whole human plasma. Upper panel depicts data from hypertriglyceridemic subjects, presented on a log scale, the line being fit to a function of the type $y = mx + b$ by least squares method. Lower panel depicts data from normotriglyceridemic subjects, a nonlog scale.

at 105,000 g at 20°C. LDL and HDL were separated by a third centrifugation, using d 1.063 g/ml saline with an 18-hr centrifugation at 105,000 g. The infranate of this spin thus included all plasma components of d > 1.063 g/ml, and are considered HDL.

Squalene was estimated using the method of Liu et al. (4) modified to measure squalene in lipoprotein fractions. All glassware, tubing, and columns used in squalene analyses were triple-rinsed with chloroform and petroleum ether (bp 30–60°C). Because human skin has a high squalene content, skin contact was strictly avoided at every step in the analysis. Three ml of whole plasma or pooled lipoprotein fraction from 6–15 ml of plasma was saponified with methanol–KOH (KOH 10% w/v, methanol 70%) in a tube containing 2 μg of squalene (Eastman Kodak Co., Rochester, NY) as a procedural standard. The nonsaponifiable material was extracted three times using 10 ml of redistilled petroleum ether (bp 30–60°C) for each extraction. This extract was applied to an 8 × 1 cm column of alumina oxide (AG 7, 200-mesh, Bio Rad Laboratory, Richmond, CA). The hydrocarbon fraction was eluted with 50 ml of petroleum ether. [^{3}H]-Cholesterol did not appear in the squalene fraction, indicating that there was no cross contamination of the hydrocarbon fraction with sterols. 5α-Cholestane (2 μg) was added as a gas–liquid chromatographic standard; the fraction was dried to 5–10 μl and applied to a 6-ft column (4 mm ID) containing 1% Dexsil-300 on 100–200 mesh Supelcoport (Supelco, Inc., Bellefonte, PA). The gas chromatograph (Model 5711 with 5830 integrator, Hewlett Packard Co., Palo Alto, CA) was run with oven temperature at 220°C. Squalene 5α-cholestane eluted with retention times of 1.58 and 1.96, respectively, relative to squalene. Radioactivity was measured by liquid scintillation counting, using Model 3255 Tricarb, (Packard Instrument Co., Inc., Downers Grove, IL). An external standard channel ratio method was used to correct for quenching.

Plasma triglyceride concentration was measured using the AutoAnalyzer Model II (Technicon, Tarrytown, NY), Method N-24. Plasma cholesterol concentration was measured using AutoAnalyzer Method N-78a, or by the method of Abell et al. (9) for concentrations of less than 50 mg/dl.

RESULTS

Whole plasma triglyceride vs. plasma squalene

In HTG subjects, a strong correlation was found between whole plasma squalene and triglyceride concentrations ($r = 0.973$, $P < 0.001$), (**Fig. 1**). In the 5 nonchylomicronemic subjects (TG 200–250 mg/dl), the squalene concentration was normal or mildly elevated (upper panel). Squalene concentration was clearly elevated in the subjects (No. 29 and 30) with plasma triglyceride concentrations of 452 and 580 mg/dl, respectively. Thus, the positive correlation of triglyceride with squalene concentration was present in the nonchylomicronemic HTG subjects ($r = 0.914$) and in the chylomicronemic subjects ($r = 0.955$). Plasma squalene concentration in all 24 NTG subjects was 19.4 ± 1.5 (mean ± SE), and did not correlate with plasma triglyceride concentration (Fig. 1, lower panel). There was no correlation between plasma squalene and cholesterol concentrations in either NTG or HTG subjects.

Rat plasma

When data from HTG (diabetic) rats and control rats were grouped, total plasma triglyceride again correlated strongly with plasma squalene ($r = 0.928$, $P < 0.001$) (**Fig. 2**). As in human plasma, this correlation was due to the progressively increased plasma

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squalene seen with progressively more severe hypertriglyceridemia. The regression line for squalene vs. triglyceride concentration in rats (Fig. 2) had a markedly different slope from that in humans (Fig. 1). Rat plasma was relatively more squalene-rich for each increment in triglyceride. Mean triglyceride and mean squalene concentrations in the control rat plasmas were higher than in the NTG human plasma (Tables 1 and 2). As in humans, there was no significant correlation between plasma squalene and cholesterol in normal rats.

Effect of diet

The effect of diet on plasma squalene and triglyceride levels is shown in **Fig. 3.** Subjects No. 10–24 are included in these data; those with fasting chylomicronemia did not show an increment of plasma triglyceride in response to diet. Fig. 3 shows that plasma triglyceride concentration rose significantly in the third hour. The rise in plasma squalene occurred after a lag period, becoming significant only at 6 hr. In three cases not reported here, administration of formula diet on a more evenly distributed schedule throughout the 24-hr period did not lead to the same rise in plasma squalene or triglyceride. This is in agreement with data collected by Liu and McNamara at The Rockefeller University on the failure of formula diet to raise plasma squalene.[2]

Lipoprotein fractionation of normal plasma

Despite the small mass of squalene in NTG plasma lipoprotein fractions, we were able to quantitate squalene by GLC with reproducible peaks. **Table 3** reports the mean squalene distribution among lipoprotein fractions in normal plasma. There was a mean procedural loss of about 5 μg/dl squalene in fractionating plasma, but mean triglyceride recovery was virtually complete. VLDL contained a higher concentration of squalene than did the other fractions; however, significant amounts of squalene were also present in LDL and HDL fractions of normal plasma. The percent squalene distribution among lipoprotein fractions closely approximated triglyceride distribution (Table 3).

[2] Liu, G. C. K., and D. J. McNamara. Personal communication.

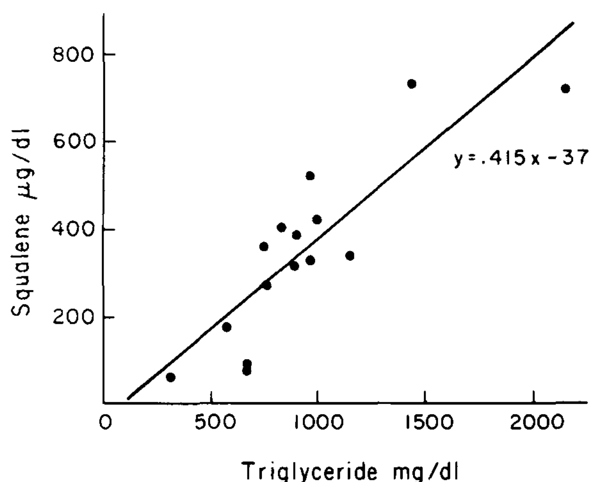

Fig. 2. Squalene vs. triglyceride: diabetic rat plasma. The line was determined by linear least squares method.

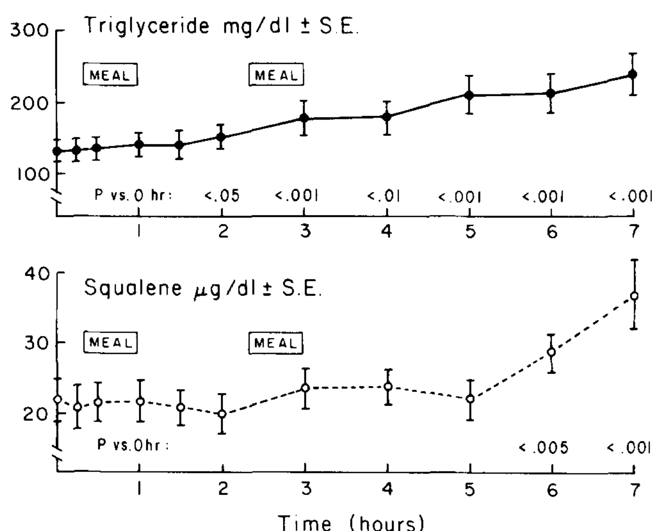

Fig. 3. Effect of diet on plasma triglyceride and squalene. Fifteen NTG subjects (No. 10–24) were studied. Time is measured from 9:30 AM, when studies were initiated. *P* denotes the significance of elevated plasma triglyceride or squalene when compared by paired control to 0 hour.

Lipoprotein fractionation of chylomicronemic plasma

With the high concentration of squalene in HTG plasma, there was no technical difficulty in quantitating squalene in the lipoprotein fractions. **Fig. 4** shows the mean percent distribution of plasma squalene and triglyceride in samples taken from four chylomicronemic subjects. While the percent of total triglyceride found in each lipoprotein fraction varied considerably from patient to patient (depending upon the degree of chylomicronemia), the percent distribution of squalene in each fraction was always within 2% of that of triglyceride. When each lipoprotein fraction's squalene concentration was plotted against triglyceride content, a significant correlation was found ($r = 0.93$, $P < 0.001$) on a regression line that was not significantly different from that found in whole plasma from HTG subjects (**Fig. 5**). Thus, squalene distribution in whole plasma or lipoprotein fractions was in close accordance to the distribution of triglyceride.

Distribution of newly synthesized [^{14}C]squalene

After administration of ^{14}C-MVA to four chylomicronemic subjects, isotopically labeled squalene

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TABLE 3. Normal human plasma squalene, triglyceride, and cholesterol concentrations in lipoprotein fractions and whole plasma

	VLDL	LDL	HDL	Sum of Fractions	Whole Plasma
Squalene (μg/dl)	9.7 ± 2.5[a]	4.9 ± 0.5	4.5 ± 0.5	19.1 ± 2.7	23.9 ± 3.5
% of sum	50.8	25.6	23.6		
Triglyceride (mg/dl)	49.6 ± 11.6	24.1 ± 1.8	30.2 ± 4.1	103.9 ± 15	107.7 ± 16
% of sum	47.7	23.2	29.1		
Cholesterol (mg/dl)	11.8 ± 3	91.8 ± 4.9	52.1 ± 5.3	155.7 ± 7.3	168.5 ± 9.1
% of sum	7.5	59.0	33.5		

[a] Mean ± SEM.

appeared in the chylomicron, VLDL, and HDL + LDL fractions (**Fig. 6**). LDL and HDL fractions were combined because of the small squalene mass and counts in each; even then, there was too little radioactivity in LDL + HDL to derive significant data in the two most chylomicronemic subjects. Considering all four subjects, the mean peak radioactivity (per counting vial) of chylomicron and VLDL extract was 241 and 615 dpm above background, respectively. The mean peak counts in the LDL + HDL extracts yielded 39 dpm. Specific activity curves were more reliable, then, in chylomicron and VLDL fractions. In all four subjects, the peak squalene specific activity of VLDL was higher than that of chylomicrons, and the fractional turnover rate of squalene in the VLDL fraction was greater than that of chylomicrons. The LDL and HDL [^{14}C]squalene, when measurable, had the most rapid turnover rate, and the total plasma squalene turnover curve in each case fell between the curves of each lipoprotein fraction. These differences, however, were not large and statistical analyses are not warranted in this small number of studies.

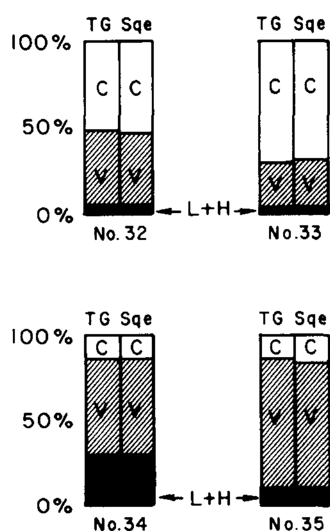

Fig. 4. Mean percent distribution of human plasma squalene (Sqe) and triglyceride (TG) among lipoprotein fractions. Plasma from four subjects (No. 32–35) is depicted. Each bar represents 100% of the whole plasma TG or Sqe, and each compartment represents the percent of TG or Sqe found in chylomicrons (*C*), VLDL (*V*), or LDL + d > 1.063 HDL (*L+H*), respectively.

Active squalene pool

The maximum pool of metabolically active plasma squalene was calculated for 15 subjects, as described previously (1, 5). This analysis is predicated upon the findings by us and others (1, 3) that the squalene specific activity in plasma dies away exponentially over the time period studied. **Table 4** presents the calculated active squalene pool sizes, which were significantly larger in seven nonchylomicronemic HTG subjects than in eight NTG subjects. In four chylomicronemic subjects, the active pool was still larger. The 10–12 hr sampling period allowed the die-away curve to be established in all subjects despite the slower turnover of [^{14}C]squalene in chylomicronemic subjects (**Fig. 6**). In each HTG group, there was a significant correlation between the calculated metabolically active plasma

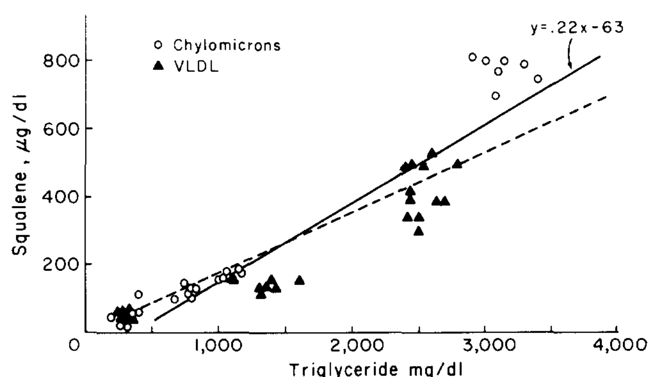

Fig. 5. Plasma squalene vs. triglyceride in human VLDL (▲) and chylomicrons (○). Data are presented from the five chylomicronemic subjects (No. 32–36), sampled on various days. Solid line is fit to a function of the type $y = mx + b$ by the method of least squares. Dotted line derived from the whole plasma data in Fig. 1. The two lines are not significantly different. d > 1.063 HDL and LDL fractions are not depicted because of their low triglyceride and squalene concentration.

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squalene pool size and the plasma triglyceride and squalene concentration (Table 4).

Peak [14C]squalene

The peak radioactivity in squalene (dpm/ml plasma) was considered for each squalene kinetic study. Twelve studies of NTG subjects had a peak of mean [14C]-squalene of 64.4 ± 8.1 dpm/ml; the corresponding value for all HTG studies was 161 ± 20.3 dpm/ml ($P < 0.001$).

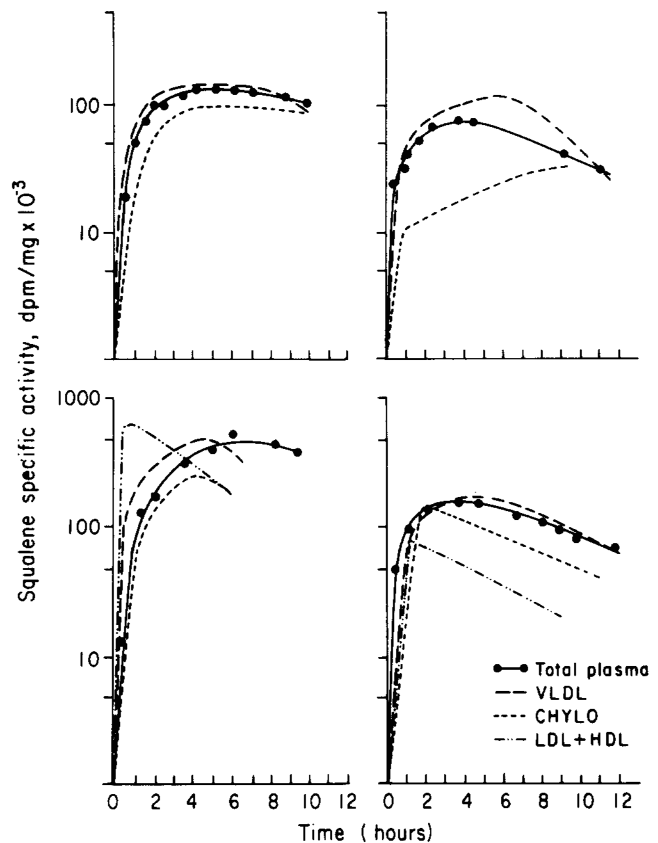

Fig. 6. Squalene specific activity curves in lipoprotein fractions. Four patients (No. 32–35) are represented, on a semi-log plot. In two patients (No. 34, 35) LDL + d > 1.063 HDL fractions had insufficient counts to derive significant data. Time is in hours after 14C-MVA administration. Data points (●) are presented for whole plasma specific activity, to indicate times of sampling.

DISCUSSION

These data show significant concentrations of squalene in each major lipoprotein fraction. The squalene concentration correlated strongly with triglyceride concentration in HTG plasma; and in NTG or HTG the squalene content of each lipoprotein fraction correlated with its triglyceride content. When experimental diabetes or diet raised plasma triglyceride, plasma squalene was also elevated. The chemical nature of the squalene–triglyceride relationship has not been studied directly, but it could be that the two lipids are independently bound to the same lipoproteins, or it could be that squalene has a nonspecific affinity for triglyceride.

The squalene and sterol carrier protein (SCP) described by Dempsey and Ritter (10, 11) and Scalen et al. (12, 13) has been stated to be present in human plasma (14) as a component of the apolipoprotein A-II (15), which is predominantly found in HDL (16, 17). It has not been demonstrated, however, that SCP, described principally in the hepatocyte, has a major role in binding plasma squalene. Our data suggest that squalene is distributed among lipoprotein fractions in a pattern not consistent with binding to ApoA-II.

We have confirmed Goodman's finding (3) that, in normal plasma, most squalene appears in the VLDL lipoprotein fraction. Our detection of significant squalene concentrations in LDL could to some extent be the result of our inclusion of the d 1.006–1.019 g/ml fraction (intermediate lipoprotein) as LDL rather than as VLDL (3). This lower density subfraction of LDL carries most of the LDL-associated triglyceride (16). However, we also found squalene in HDL and

TABLE 4. Calculated metabolically active plasma squalene pool size in normotriglyceridemia, nonchylomicronemic hypertriglyceridemia, and chylomicronemia

Subjects	(n)	Calculated Pool Size (mg)	P^a	Correlation of Pool Size vs. Squalene r	Correlation of Pool Size vs. Squalene P^b	Correlation of Pool Size vs. Triglyceride r	Correlation of Pool Size vs. Triglyceride P^b
Normotriglyceridemia	(8)	232 ± 22[c]		0.10	ns[d]	0.08	ns
Nonchylomicronemic hypertriglyceridemia	(7)	457 ± 98	<0.05	0.975	<0.001	0.964	<0.001
Chylomicronemic hypertriglyceridemia	(4)	3481 ± 1533	<0.02	0.99	<0.001	0.947	<0.02

[a] P compares the pool size in hypertriglyceridemic groups with that in normotriglyceridemia.
[b] P denotes the significance of the correlation (r) of pool size vs. squalene or triglyceride concentration.
[c] Mean ± SEM.
[d] Not significant.

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chylomicrons when they were present. The demonstration that [^{14}C]squalene appears in all lipoprotein fractions after injection of ^{14}C-MVA indicates that squalene in each of these fractions is derived at least in part from an active pool of MVA. Furthermore, the lipoprotein turnover curves do not indicate that squalene associated with LDL is derived from that squalene associated with VLDL as it is catabolized to LDL. The precursor–product relationship is not compatible with the data. VLDL and LDL + HDL squalene turnover rates were more rapid than established lipoprotein turnover times. This suggests that newly synthesized squalene is equilibrating on and off lipoprotein fractions. We cannot determine whether squalene transfers from one lipoprotein fraction to another, although the differences among turnover curves suggest that complete equilibration is not achieved.

Our data indicate that the rise in squalene follows closely the rise in plasma triglyceride. Thus diet-induced hypertriglyceridemia is followed by hypersqualenemia within 3 hr; and the experimental diabetic lipemia in rats, 48 hr after streptozotocin administration, was associated with greatly elevated plasma squalene. Since the diets were virtually squalene-free in both cases, it may be concluded that diet was not a significant source of plasma squalene. The rapid accumulation of squalene in plasma could theoretically result from increased HMG-CoA reductase activity with increased squalene production and failure to correspondingly increase squalene utilization in cholesterol synthesis. The fact that [^{14}C]squalene counts per ml plasma were higher in the HTG groups, despite the expanded pool size, is consistent with enhanced squalene production. It is possible that the enzymes responsible for squalene cyclization (squalene epoxidase and 2,3-oxidosqualene sterol cyclase) are saturated in the setting of HTG.

Hydrophobic binding might be expected between the nonpolar squalene molecule and triglyceride in the plasma. If this affinity continuously draws cold squalene from an inactive or slowly turning over pool (such as adipose tissue squalene), then the plasma kinetics of isotopically labeled squalene, newly formed from MVA, would not reflect intracellular cholesterol synthesis. If, on the other hand, the plasma squalene pool is expanded only by newly formed squalene, then while the fractional turnover rate of [^{14}C]squalene would be diminished, the absolute turnover rate would accurately reflect cholesterol synthesis.

Plasma squalene concentration itself has been said by Miettinen (17) to reflect cholesterol synthesis. He found that cholestyramine feeding raised plasma squalene concentration as it raised cholesterol synthesis, although he later questioned this relationship (18). Nestel and Kudchodkar (19) also noted a relationship between plasma squalene concentration and cholesterol synthesis. If plasma squalene does qualitatively reflect cholesterol synthesis, our documentation of hypersqualenemia in HTG could be considered consistent with the purported high cholesterol synthesis in HTG (20–22). Grundy (23), however, argued that HTG is not uniformly associated with increased cholesterol synthesis. In distinction to the small and variable increase in cholesterol synthesis found in HTG patients, the plasma squalene concentration is consistently and often massively elevated.

Squalene kinetic analysis as originally proposed (1, 2, 4) is quantitatively accurate if the plasma squalene pool is in equilibrium with active intracellular squalene pools. We have demonstrated that each major lipoprotein fraction in chylomicronemic plasma contains metabolically active squalene, and that whole plasma squalene kinetics represent a composite of those individual turnover curves. Squalene kinetic analysis has been in agreement with sterol balance data in several HTG subjects studied by Liu et al. (1) and McNamara et al. (2), but more studies will be necessary to determine whether chylomicrons, in particular, affect squalene kinetics sufficiently to alter the estimation of cholesterol synthesis. ■

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