# Fate of intravenously administered squalene and plant sterols in human subjects

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**Abstract**   We have studied metabolism of plant sterols and squalene administered intravenously in the form of lipid emulsion mimicking chylomicrons (CM). The CM-like lipid emulsion was prepared by dissolving squalene in commercially available Intralipid®. The emulsion was given as an intravenous bolus injection of 30 ml containing 6.3 mg of cholesterol, 1.9 mg of campesterol, 5.7 mg of sitosterol, 1.6 mg of stigmasterol, 18.1 mg of squalene, and 6 g of triglycerides in six healthy volunteers. Blood samples were drawn from the opposite arm before and serially 2.5–180 min after the injections. The decay of CM squalene, plant sterols, and triglycerides was monoexponential. The half-life of CM squalene was $74 \pm 8$ min, that of campesterol was $37 \pm 5$ min ($P < 0.01$ from squalene), and those of sitosterol, stigmasterol, and triglycerides were $17 \pm 2$, $15 \pm 1$, and $17 \pm 2$ min, respectively ($P < 0.01$ from squalene and campesterol). The CM squalene concentration still exceeded the baseline level 180 min after injection ($P = 0.02$), whereas plant sterols and triglycerides returned to the baseline level between 45 and 120 min after injection. The half-lives of squalene and campesterol were positively correlated with their fasting CM concentrations. In addition, VLDL squalene, campesterol, and triglyceride concentrations, VLDL, LDL, and HDL sitosterol concentrations, as well as VLDL and LDL stigmasterol concentrations were increased significantly. Cholesterol concentrations increased in VLDL ($P < 0.05$), but were unchanged in CM after injection.▮▮ These data suggest that squalene clearance occurs more slowly than that of plant sterols and triglycerides from CM, and that squalene is more tightly associated with triglyceride-rich lipoproteins than are plant sterols in injected CM-like emulsions. —Relas, H., H. Gylling, and T. A. Miettinen. **Fate of intravenously administered squalene and plant sterols in human subjects.** *J. Lipid Res.* 2001. **42:** 988–994.

**Supplementary key words**   campesterol • sitosterol • stigmasterol • cholesteryl esters • triglycerides • chylomicrons • Intralipid

Triglyceride-rich lipoproteins (TRL) are a heterogeneous group of lipoproteins that originate from small intestine and liver. The liver secretes VLDL, which contain apolipoprotein B-100 (apoB-100) as their structural protein. On the other hand, dietary fat and cholesterol are carried from the small intestine into the blood stream by chylomicrons (CM), which contain apoB-48 as their structural protein. Triglycerides are lipolyzed by LPL in the circulation. The remaining particles are known as CM remnants, which are rich in cholesterol. CM remnants have the same particle size and density as VLDL produced by the liver, and they can be determined on the basis of apoB-48 and apoB-100 (1), or indirectly by orally fed markers such as retinyl palmitate (2) or squalene (3). An intravenous fat tolerance test using Intralipid® as an artificial CM-like lipid emulsion has been suggested as a possible tool with which to study lipid metabolism, because the metabolism of Intralipid® mimics the metabolism of endogenous TRL (4). For this purpose, CM-like emulsions have been labeled with [14C]cholesteryl ester and [3H]triolein also in human studies (5, 6).

Because the human body is unable to synthesize plant sterols (7), their low serum concentrations originate from dietary plant materials. Three consecutive daily infusions of 500 ml of Intralipid®, which is rich in plant sterols, to a patient receiving parenteral nutrition increased markedly plasma sitosterol concentrations, which were normalized in about 15 days (8). The kinetics and lipoprotein distribution in humans of various plant sterols after an intravenous administration are not known. In rats, [14C]cholesterol and [3H]sitosterol administered intravenously with Intralipid® appeared rapidly, with CM and VLDL, and also with LDL and HDL (9). In addition, the clearance rates of intravenously given cholesterol and plant sterols are similar, at least in the rat (10).

Squalene, a nonpolar, nonsterol cholesterol precursor, has been used as a marker for postprandial lipoproteins (3, 11), but its metabolism after an intravenous injection has not been studied in humans. However, after an intravenous injection of [14C]mevalonic acid, plasma squalene becomes rapidly labeled, and is converted to cholesterol (12, 13). In the rat, intravenously administered [3H]squalene dis-

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TABLE 1. Mean fasting concentrations of cholesterol, plant sterols, squalene, and triglycerides in serum and lipoproteins of study population

| | Serum | CM | VLDL | IDL | LDL | HDL |
|---|---|---|---|---|---|---|
| Cholesterol (mg/dl) | 193.8 ± 14.8 | 4.7 ± 0.97 | 6.55 ± 1.07 | 3.7 ± 0.70 | 104.8 ± 10.4 | 53.9 ± 3.6 |
| Campesterol (μg/dl) | 623.5 ± 99.6 | 14.1 ± 2.4 | 20.7 ± 4.7 | 11.3 ± 2.3 | 331.5 ± 46.3 | 186.0 ± 30.0 |
| Sitosterol (μg/dl) | 319.5 ± 40.1 | 7.8 ± 1.6 | 7.9 ± 1.3 | 5.3 ± 1.0 | 154.6 ± 16.3 | 99.3 ± 15.0 |
| Stigmasterol (μg/dl) | 16.2 ± 8.5 | 0.52 ± 0.14 | 0.57 ± 0.23 | 0.32 ± 0.07 | 5.9 ± 2.5 | 5.5 ± 3.2 |
| Squalene (μg/dl) | 144.7 ± 26.4 | 7.1 ± 0.77 | 16.6 ± 3.5 | 7.8 ± 2.8 | 62.0 ± 16.0 | 51.2 ± 10.8 |
| Triglycerides (mg/dl) | 75.9 ± 3.7 | 7.5 ± 1.1 | 30.8 ± 4.9 | 4.4 ± 0.6 | 15.3 ± 1.5 | 10.8 ± 1.1 |

Values represent means ± SE, n = 6. CM, chylomicrons.

appeared multiexponentially, and the rate of disappearance was faster than that of [14C]cholesterol (14).

We considered whether a study of the clearance of the plant sterols of Intralipid® with added squalene would be comparable to that of postprandial lipoproteins. To our knowledge, the removal of plant sterols and squalene of CM-like emulsions has not been studied in humans. Thus, the aim of the present study was to investigate the lipoprotein clearance of squalene, plant sterols (campesterol, sitosterol, and stigmasterol), and triglycerides after an intravenous injection of Intralipid® enriched with squalene in healthy human subjects.

## MATERIALS AND METHODS

### Study population

Six healthy men aged 40 ± 7 years (mean ± SE) participated in the Intralipid® clearance test. The subjects were nonobese (body mass index, 22.1 ± 0.5 kg/m²), normolipidemic (**Table 1**) volunteers, and each had an apoE-3/3 phenotype. The study protocol had been approved by the Ethics Committee of our hospital, and the subjects gave their informed consent.

### Preparation of the emulsion containing squalene

CM-like lipid emulsion containing squalene, called squalene emulsion, was prepared by dissolving squalene into commercially available Intralipid® 200-mg/ml fat emulsion (Pharmacia, Stockholm, Sweden) as follows. Liquid 98–100% squalene (Sigma, St. Louis, MO) was first filtered with a sterile Millex-GV 0.22 μm pore size filter unit (Millipore, Molsheim, France) to ensure its sterility. To make squalene soluble, 80 mg of squalene was dissolved in 0.9 ml of 99.5% ethanol (Apoteksbolaget, Umeå, Sweden), and the squalene–ethanol 1:9 (v/v) solution was subsequently dissolved in 100 ml of Intralipid®.

### Study protocol

The subjects were admitted to the laboratory in the morning after an overnight fast. After the baseline blood sample had been obtained, 30 ml of squalene emulsion was injected as an intravenous bolus into a forearm vein over 2–3 min. The samples were collected from the opposite side into 10 ml tubes (Venoject Autosep®; Terumo Europe, Leuven, Belgium) via an intravenous cannula (Venflon2® 1.2/45 mm cannula; BOC Ohmeda, Helsingborg, Sweden) 2.5, 5, 10, 20, 30, 45, 60, 120, and 180 min after the injection. No side effects occurred. As a control, 30 ml of saline was injected into two subjects, and the samples were drawn in the same manner.

### Analytical methods

Commercial kits were used to enzymatically analyze serum and lipoprotein cholesterol, triglycerides (Roche Diagnostics, Hoffman-La Roche, Basel, Switzerland), and phospholipids (Wako Chemi-

cals, Neuss, Germany). ApoE phenotyping was performed by isoelectric focusing from serum (15). Lipoproteins were separated by ultracentrifugation in a fixed-angle Beckman (Fullerton, CA) rotor into density classes (16). CM were separated after a 30-min ultracentrifugation with carefully overlayered 1.006-g/ml NaCl salt solution, and the other lipoproteins were separated according to densities as follows: VLDL, <1.006 g/ml; IDL, 1.006–1.019 g/ml; LDL, 1.019–1.063 g/ml; and HDL, 1.063–1.210 g/ml.

Serum cholesterol, squalene, and noncholesterol sterols (cholestanol, Δ8-cholestenol, desmosterol, lathosterol, and plant sterols campesterol, stigmasterol, and sitosterol) were quantitated by gas-liquid chromatography (GLC) on a 50-m-long Ultra 1® SE-30 column (Hewlett-Packard, Palo Alto, CA) from saponified serum or lipoprotein fractions as their trimethylsilyl derivatives, using 5α-cholestane as an internal standard (17, 18). Free and esterified cholesterol and plant sterols from Intralipid® and CM samples were quantitated by GLC after the free and esterified sterols had been separated by thin-layer chromatography as described previously (18).

### Calculations

CM squalene, plant sterol, and triglyceride clearance curves are presented separately for each subject. The data are presented as concentrations or incremental concentrations by subtracting the fasting concentration from the concentration at the respective time point. In addition, die-away curves expressing the percentage of peak squalene, plant sterol, and triglyceride concentration in CM were constructed.

### Statistical analysis

Data analyses were performed by the Biomedical Data Program (BMDP Statistical Software, Los Angeles, CA). Statistical significance was tested by ANOVA for repeated measures, one-way ANOVA, and a two-sided Student's *t*-test and paired *t*-test. Correlations were analyzed by calculating Spearman's correlation coefficient. Half-lives of CM squalene, plant sterols, and triglycerides were determined by nonlinear least-squares analysis. Logarithmic transformations were performed with skewed distributions. A *P* value <0.05 was considered significant.

## RESULTS

The original concentration of squalene in Intralipid® was 0.80 ± 0.12 mg/dl. After addition of squalene, its concentration in the emulsion was 60.3 ± 1.4 mg/dl. The emulsion used in the experiments contained cholesterol at 21.0 mg/dl, campesterol at 6.4 mg/dl, sitosterol at 19.0 mg/dl, and stigmasterol at 5.3 mg/dl. Thus, the dose given by an injection of 30 ml of squalene emulsion contained, on average, 18.1 mg of squalene, 6.3 mg of cholesterol, 5.7 mg of sitosterol, 1.9 mg of campesterol, and 1.6

TABLE 2. Percentage of esterified cholesterol and plant sterols of Intralipid®, and percent distribution of cholesterol, plant sterols, and squalene in various density classes after enrichment of Intralipid® with squalene

|  | CH | CA | SI | ST | SQ |
|---|---|---|---|---|---|
| Esterified (%) | 1 | 12 | 25 | 6 | — |
| d <0.95 (%) | 73 | 83 | 86 | 80 | 84 |
| d 0.95–1.006 (%) | 8 | 4 | 6 | 8 | 13 |
| d 1.006–1.063 (%) | 16 | 12 | 7 | 11 | 2 |
| d 1.063–1.210 (%) | 3 | 1 | <1 | <1 | 1 |

Abbreviations: CH, cholesterol; CA, campesterol; SI, sitosterol; ST, stigmasterol; SQ, squalene.

mg of stigmasterol. The dose of injected triglycerides was 6 g (0.08–0.10 g/kg body weight). Only 1% of Intralipid® cholesterol was esterified, whereas the respective values for plant sterols were 6–25% (**Table 2**). Ultracentrifugation of the squalene emulsion revealed that the majority of sterols and squalene were detected at d < 0.95 g/ml, but small amounts were found also at d 0.95–1.063 g/ml (Table 2).

Fasting serum lipid concentrations are shown in Table 1. More than 70% of serum squalene and plant sterols were carried in LDL and HDL, whereas triglycerides were found mainly in VLDL.

## CM lipids

The injection did not significantly affect CM cholesterol concentrations (data not shown), because the amount of injected cholesterol was small compared with the fasting concentrations of CM cholesterol (Table 1). Peak CM concentrations of sitosterol and triglycerides in every subject were observed at 2.5 min, as well as campesterol and stigmasterol in five and squalene in four subjects (**Fig. 1**). The decay of CM squalene, plant sterols, and triglycerides was monoexponential, and no improvement could be achieved by introducing more exponents to data fitting. Squalene was cleared more slowly than plant sterols and triglycerides from CM (**Fig. 2**). The CM squalene concentration (27.7 ± 6.0 µg/dl) at 180 min was still significantly above the baseline ($P = 0.02$), and its half-life was significantly longer than that of plant sterols and triglycerides (**Table 3**). The clearance rate of CM campesterol was slower than those of other plant sterols and triglycerides (Fig. 2), resulting also in its longer half-life (Table 3). Sitosterol, stigmasterol, and triglycerides exhibited a similar pattern of clearance in CM (Fig. 2), and their baseline levels were reached between 60 and 120 min (Fig. 1). CM campesterol increments were about 20% of sitosterol increments because the injected dose of campesterol was smaller and its baseline concentration was higher compared with sitosterol. There were associations of fasting
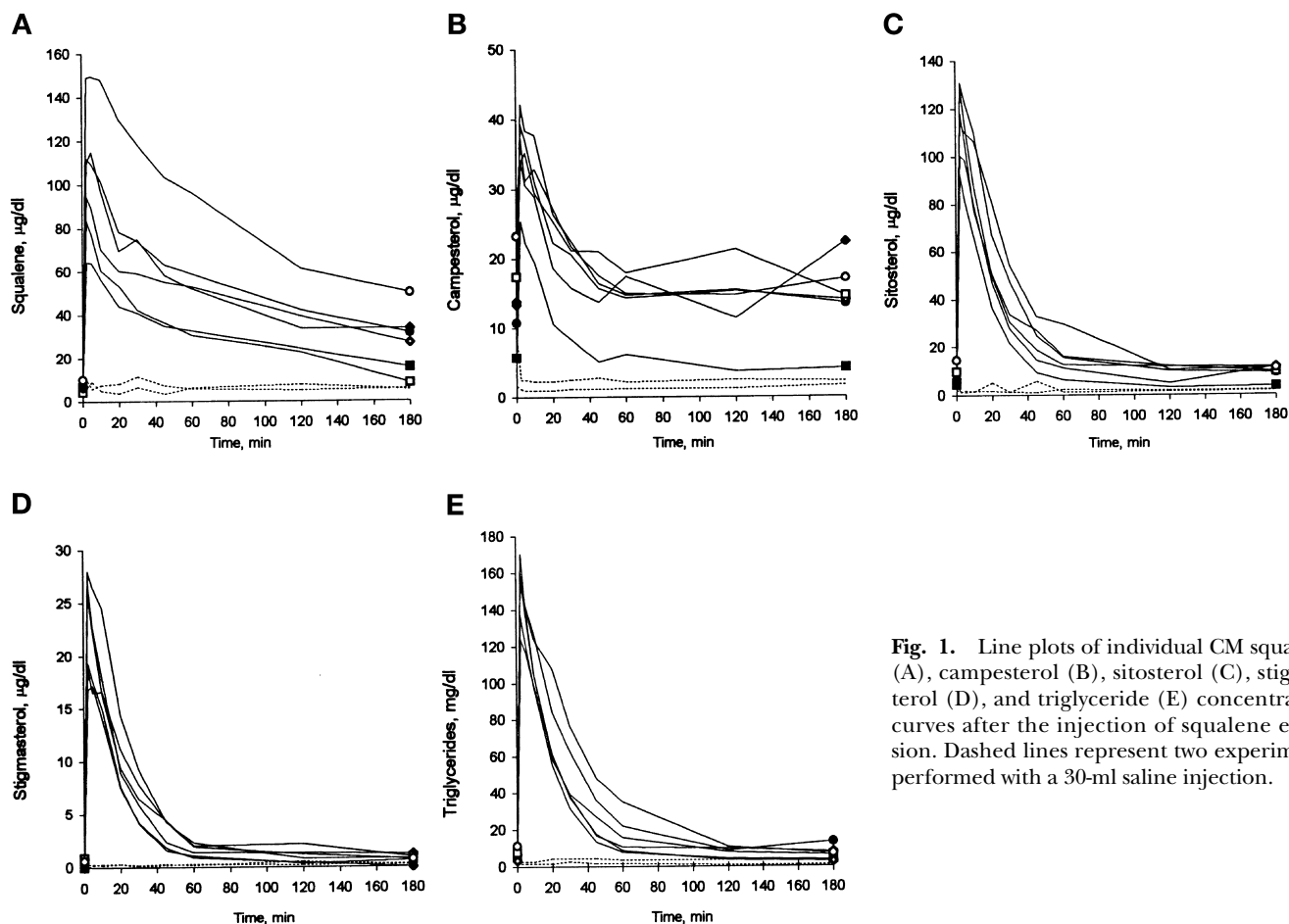


**Fig. 1.** Line plots of individual CM squalene (A), campesterol (B), sitosterol (C), stigmasterol (D), and triglyceride (E) concentration curves after the injection of squalene emulsion. Dashed lines represent two experiments performed with a 30-ml saline injection.
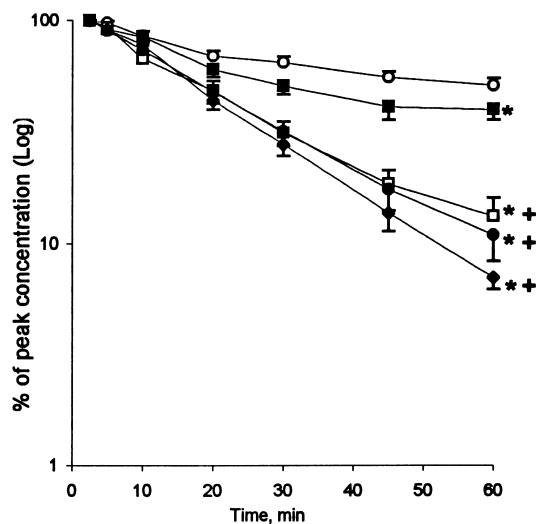
**Fig. 2.** CM squalene (open circles), campesterol (solid squares), sitosterol (open squares), stigmasterol (solid diamonds), and triglyceride (solid circles) disappearance curves after the injection of squalene emulsion. * $P \leq 0.01$ from squalene; + $P < 0.01$ from campesterol (ANOVA for repeated measures). Results represent the means ± SE of six subjects.

CM concentrations of squalene ($r = 0.899$, $P < 0.05$), of campesterol ($r = 0.841$, $P < 0.05$), and less consistently of sitosterol ($r = 0.600$, NS) with their half-lives.

The percentages of esterified CM sitosterol, campesterol, and stigmasterol of total in the fasting state were 47 ± 3%, 56 ± 5%, and 35 ± 6%, respectively (NS). Concentrations of unesterified and esterified sitosterol in CM increased almost equally although 75% of injected sitosterol was unesterified, and these two forms of sitosterol were, however, removed similarly from CM after the injection (**Fig. 3**). Similarly, the proportions of esterified campesterol and stigmasterol were not significantly altered after the injection (ranging from 45% to 50% for campesterol and from 24% to 33% for stigmasterol).

### VLDL and IDL lipids

Incremental concentrations of cholesterol, squalene, plant sterols, and triglycerides (**Fig. 4**) increased rapidly in

TABLE 3. Half-lives of squalene, plant sterols, and triglycerides in chylomicrons after the injection of squalene emulsion

| | $t_{1/2}$ | |
|---|---|---|
| | Mean ± SE | Range |
| | *min* | |
| Squalene | 74.1 ± 7.9[a,b] | 44–95 |
| Campesterol | 36.9 ± 5.0[c] | 19–55 |
| Sitosterol | 17.4 ± 2.0 | 13–27 |
| Stigmasterol | 15.1 ± 1.4 | 12–21 |
| Triglycerides | 17.2 ± 2.3 | 12–27 |

Results represent means ± SE, n = 6.
[a] $P = 0.003$ from campesterol; one-way ANOVA.
[b] $P < 0.001$ from sitosterol, stigmasterol, and triglycerides; one-way ANOVA.
[c] $P < 0.01$ from sitosterol, stigmasterol, and triglycerides; one-way ANOVA.
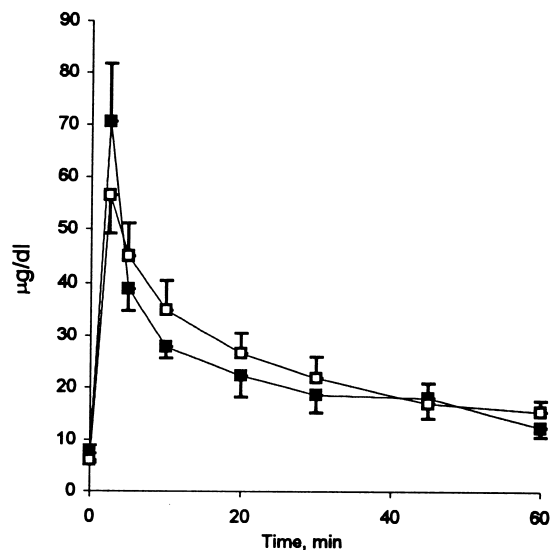


**Fig. 3.** Removal curves of unesterified (solid squares) and esterified (open squares) sitosterol in CM. $P = 0.37$, ANOVA for repeated measures. Results represent means ± SE of six subjects.

VLDL after the injection. The increments of squalene, sitosterol, and stigmasterol were significantly different from baseline from 2.5 min up to 120 min ($P < 0.03$), whereas those of campesterol and triglycerides were significant only up to 60 min ($P < 0.05$). VLDL cholesterol increments were significant at 30–60 min ($P < 0.05$). In contrast to sitosterol ($P = 0.03$) and stigmasterol ($P = 0.01$), the other lipids were unchanged in IDL (data not shown).

### LDL and HDL lipids

LDL and HDL sitosterol, and LDL stigmasterol concentrations were significantly increased after the injection (Fig. 4). The increments of sitosterol were significant from 2.5 to 180 min ($P < 0.05$), except at 20 min in LDL and at 120 min in HDL. The increments of LDL stigmasterol were significant at 2.5 min ($P = 0.03$) and at 5 min ($P = 0.008$). Squalene, campesterol, and triglyceride concentrations were not significantly altered in LDL and HDL after the injection (data not shown). LDL and HDL cholesterol concentrations were initially significantly decreased at 2.5–5 min ($P < 0.05$), and then returned to the baseline levels. However, similar LDL and HDL cholesterol configurations were observed in saline control experiments (data not shown).

### DISCUSSION

CM-like emulsion particles are cleared in two steps, like CM. Triglycerides are first removed by the lipolytic action of LPL, and the remaining remnant particles rich in cholesterol are then removed from circulation by the liver. The process of CM remnant and VLDL uptake includes endocytosis by hepatocytes, and the process is mediated by LDL receptor, LDL receptor-related protein, and cell-surface heparan sulfate proteoglycans (19). ApoE is necessary to mediate receptor binding of large TRL particles
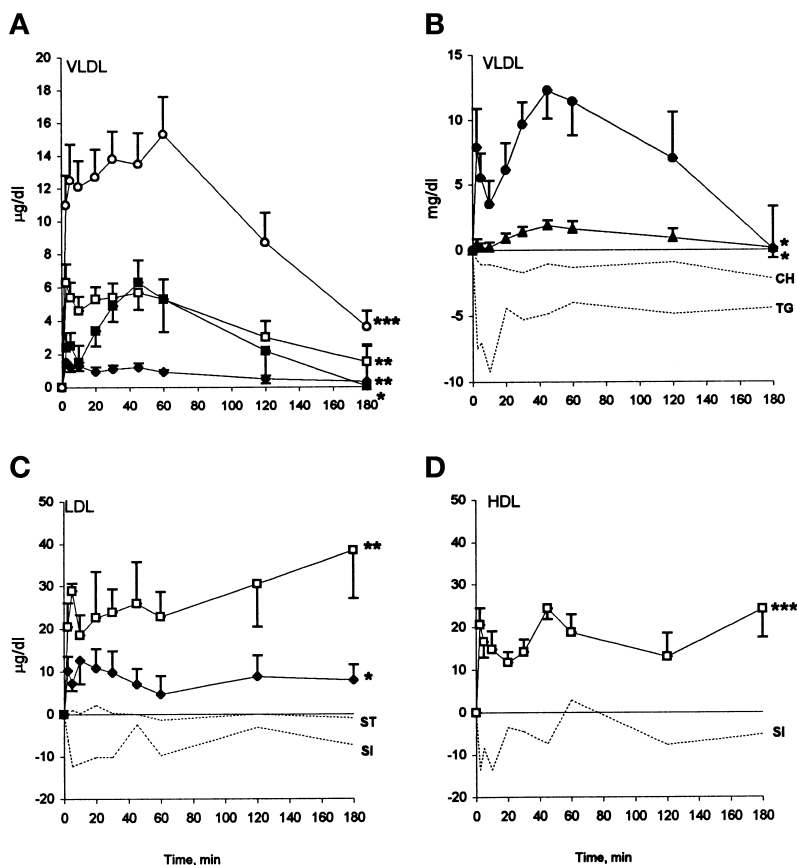
**Fig. 4.** Incremental concentration curves of squalene (open circles), campesterol (solid squares), sitosterol (open squares), and stigmasterol (solid diamonds) in VLDL (A), cholesterol (solid triangles) and triglycerides (solid circles) in VLDL (B), sitosterol and stigmasterol in LDL (C), and sitosterol in HDL (D) after the injection of squalene emulsion. Significant concentration changes from the baseline are indicated for individual concentration curves: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (ANOVA for repeated measures). Results represent means ± SE, n = 6. Control curves (dotted lines) represent mean values of two experiments performed with a 30-ml injection of saline. CH, Cholesterol; SI, sitosterol; ST, stigmasterol; TG, triglycerides. VLDL campesterol, squalene, SI, and ST control curves showed a configuration comparable to that of the CH control curve (data not shown).

to the LDL receptor (20). CM-like emulsions are cleared similarly (4, 21, 22), or somewhat slower (23), than CM. The presence of unesterified cholesterol seems to be necessary for the efficient clearance of CM-like emulsions (10, 22). In addition, it has been shown that the elimination of triglycerides follows first-order kinetics after intravenous doses less than 0.2 g/kg body weight (4, 24), which is in agreement with the present results.

Intravenously administered CM-like emulsions adsorb apoC-II from circulating lipoproteins (25), and activate LPL (26–27), phospholipid transfer protein (PLTP), CETP, and LCAT (28). Dietary fat intake acutely increases both CM remnants and VLDL (29, 30), the latter because of competition with hepatic uptake. Similarly, infusions of CM-like triglyceride emulsions cause accumulation of VLDL of hepatic origin, because they are degraded by the same pathways (31). Studies related to intravenous CM or CM-like emulsions are mostly rat (9, 10, 21–23, 26, 27, 32) or mouse experiments (33), but human studies have also been performed (4–6, 8, 31, 34). For example, patients with coronary artery disease have retarded clearance of [14C]cholesteryl ester after an injection of CM-like lipid emulsion (5).

It was assumed that squalene and plant sterols would be cleared more slowly than triglycerides, because the clearance of labeled cholesteryl esters is slower than that of labeled triglycerides injected with CM-like emulsions in humans (5, 6, 34), suggesting that cholesteryl esters remain in the core of remnant particles until taken up by the liver.

Squalene and campesterol were cleared more slowly than triglycerides, suggesting their association with particles that remain in circulation after lipolysis. Sitosterol and stigmasterol, however, were removed from CM more rapidly than expected, at a rate comparable to triglycerides. In addition, squalene, campesterol, and triglyceride concentrations were increased only in CM and VLDL after the injection of squalene emulsion, whereas sitosterol and stigmasterol appeared also in LDL, and sitosterol even in HDL (Fig. 4).

Because the concentrations of VLDL plant sterols, squalene, and triglycerides had already increased markedly at 2.5 min in the present study, they can be assumed to originate directly from the emulsion particles of smaller size. On the other hand, the increase in these lipids in VLDL after their initial decreases at 5–10 min (Fig. 4) could be derived from CM remnants, although a large fraction of the Intralipid® emulsion particles is removed with little or no lipolysis from the circulation (23). In fact, [3H]sitosterol incorporated in Intralipid® and administered intravenously to rats (9) produced results similar to our study, including a similar pattern of CM sitosterol clearance as well as the rapid appearance of sitosterol in VLDL, LDL, and HDL.

Because the plant sterols of squalene emulsion were mainly in unesterified form (Table 2), it is probable that the injected unesterified sterols are surface associated in the particles together with phospholipids, thus predisposing to plasma proteins. The plasma lipid transfer proteins mediate the transfer and exchange of phospholipids and

neutral lipids between the plasma lipoproteins. After hydrolysis of Intralipid® TRL, surface remnant lipids composed largely of phospholipids are transferred to HDL (35). LCAT, which catalyzes the formation of cholesteryl esters in human plasma (36), is also capable of esterifying sitosterol (37). CETP facilitates the exchange of neutral lipids between the plasma lipoproteins, and cholesteryl ester and triglyceride transfer activities are determined by the concentrations of these lipids at the surface of donor and acceptor particles (38). PLTP facilitates the transfer and exchange of phospholipids but not the exchange of neutral lipids between lipoproteins (39), and HDL is probably the most efficient phospholipid acceptor in PLTP-mediated transfer reactions (40). PLTP has also been suggested to enhance the transfer of unesterified cholesterol from phosphatidylcholine- and cholesterol-containing bilayer vesicles to HDL (41).

Thus, it is possible that the rapid clearance of CM sitosterol and stigmasterol, and the appearance of sitosterol, in HDL is related to the activity of lipid transfer proteins, or is an unspecific transfer process due to concentration gradients. Rapid initial esterification of cholesterol after an intravenous injection, described earlier by Viikari et al. (32), could explain the modest increase in unesterified sitosterol in relation to the injected dose (Fig. 3). On the other hand, increases in LDL sitosterol and stigmasterol in the present study can partly be explained by their presence (7–11% of total) in squalene emulsion particles of LDL density. The previous finding that squalene disappears faster than cholesterol after an intravenous injection from rat serum, partly cyclizes to sterols, and partly re-enters the circulation (14) could not be observed in the present human studies. The changes in squalene concentrations only in d < 1.006 g/ml particles after the injection suggest that as a nonpolar hydrocarbon it is not detectably transferred between the lipoprotein fractions.

Campesterol has a methyl group and sitosterol has an ethyl group at the C-24 position, which are their only differences from cholesterol. Thus, campesterol is more polar than sitosterol (42). A C-22 double bond in stigmasterol is the only difference from sitosterol. The clearance of CM-like emulsion requires the presence of a hydroxyl group at the 3-position and an alkyl side chain at the C-17 position of cholesterol, but the side chain at the C-24 position does not affect clearance in the rat (10). However, after simultaneous pulse labeling with [$^3$H]sitosterol and [$^{14}$C]cholesterol by the intravenous route, the half-life of sitosterol was shorter than that of cholesterol studied in human plasma (43), suggesting that the removal of different sterols may vary in humans. Because 4–5% of sitosterol (43, 44) and about 10% of campesterol are absorbed (44), it is possible that the higher serum concentrations of campesterol compared with sitosterol (45) are explained by its better absorption.

The present results suggest that the clearance of campesterol might be slower than that of sitosterol and stigmasterol in humans. Campesterol, being more polar than sitosterol, should be more surface associated and thus more rapidly removed than sitosterol if the phenomenon were dependent on the polarities. However,

the slower campesterol clearance suggests that esterified campesterol may remain longer in the core of emulsion remnants. This could be possible if campesterol and sitosterol were rapidly esterified to different extents after injection, as 12% of campesterol and 25% of sitosterol in the emulsion were esterified. On the other hand, the lower emulsion concentration and the higher basal lipoprotein concentration of campesterol compared with sitosterol may prevent the detection of any changes in campesterol concentrations in LDL and HDL. The relationship between fasting CM campesterol concentrations and the half-lives after injection suggests that the higher baseline concentration of campesterol may contribute to its slower clearance compared with sitosterol.

The major observation of the present study was that the clearance rate of CM squalene after an intravenous injection of CM-like squalene emulsion is slower than that of campesterol, and also is slower than those of triglycerides, sitosterol, and stigmasterol, three lipids with identical rates of removal. In addition, new data about plant sterols and squalene in various lipoproteins after intravenous administration in human subjects are provided, showing, for example, that removal of those lipids depends on their fasting concentrations. Because squalene labels postprandial lipoproteins with higher specificity than vitamin A as compared with postprandial apoB-48 levels (11), squalene could be used to measure postprandial lipoproteins also added to CM-like emulsions, giving the advantage of avoiding the use of radioactive labels. The same degree of accuracy, however, cannot be achieved compared with methods using isotope labels. Because the present study was not designed to explore the mechanisms by which plant sterols are metabolized in various lipoproteins, the metabolism of intravenous plant sterols needs to be further investigated in human subjects.▊

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