Dietary squalene increases cholesterol synthesis measured with serum non-cholesterol sterols after a single oral dose in humans

Heikki Relas, Helena Gylling, Tatu A. Miettinen *

Department of Medicine, Division of Internal Medicine, University of Helsinki, PO Box 340, 00029 HYKS, Helsinki, Finland

Received 25 June 1999; received in revised form 14 October 1999; accepted 2 November 1999

Abstract

Studies considering long-term squalene consumption have revealed no consistent effects on serum cholesterol levels but the immediate effect of dietary squalene on cholesterol synthesis has not been studied. Thus, the effect of a single dose of dietary squalene on postprandial lipid metabolism was studied in 16 male volunteers aged 22–79 years. Two oral fat meals a week apart were administered to every subject, one without (control) and the other with 500 mg of squalene. Lipids, retinyl palmitate, squalene and non-cholesterol sterols were measured at baseline and after 3, 4, 6, 9, 12 and 24 h postprandially in plasma, chylomicron, VLDL and VLDL bottom and, in six randomly chosen subjects, also in IDL, LDL and HDL. In the fasting samples, squalene was mainly transported in LDL and HDL, whereas in squalene-supplemented postprandium most of squalene was carried in the triglyceride-rich lipoproteins. Postprandial squalene and retinyl palmitate curves closely resembled each other. After the squalene-enriched dietary fat load, squalene was significantly increased compared to control fat loads in plasma, chylomicrons, VLDL and IDL. Squalene addition increased significantly lathosterol/campesterol ratio in chylomicrons and VLDL at 12 h and in VLDL bottom at 9–12 h, and increased significantly VLDL lanosterol/campesterol ratio at 12 h, indicating enhanced cholesterol synthesis caused by squalene. Plasma plant sterol levels remained unchanged. In conclusion, a single oral dose of squalene representing a potential daily dietary amount increases cholesterol synthesis within 9–12 h detected in chylomicrons, VLDL and VLDL bottom. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Squalene; Cholesterol synthesis; Triglycerides; Lathosterol; Methyl sterols; Plant sterols; Postprandial lipids

1. Introduction

Squalene is a 30-carbon and 50-hydrogen non-steroid intermediate of cholesterol synthesis preceding the formation of steroid nucleus. Skin and adipose tissue are rich in squalene in humans, whereas serum contains relatively low levels of squalene [1]. Serum squalene is rapidly equilibrated with the squalene pool in liver [2], and kinetic analyses have shown that serum squalene has a rapid turnover to cholesterol [2–6]. In fact, serum squalene concentration has been considered as an indicator of cholesterol synthesis rate [7,8] even though not as consistent as the other sterol precursors of cholesterol, i.e. Δ5-cholestenol, desmosterol and lathosterol [9]. Dietary squalene is readily absorbed such that the serum concentration reflects also its dietary intake [1,10,11]. Squalene is transported postprandially in chylomicrons and chylomicron remnants so that its postprandial serum concentration can be used to elucidate the rate of fat clearance [12,13]. It has been approximated that, e.g. in the USA, squalene intake averages 100 mg/d [1], but in populations consuming large amounts of olive oil the daily intake can be much higher [1]. High dietary intake of squalene, ranging from 850 to 1000 mg/day, has variable effects on serum cholesterol level ranging from lowered [14] through unchanged [10] to increased values [11]. However, long-term squalene intake increases cholesterol synthesis rate measured with serum cholesterol precursor sterols [10,11] and also methyl sterols [11]. The question now arises what is the effect of a single oral dose of squalene combined with fat on postprandial cholesterol metabolism. Accordingly, we examined changes in cholesterol precursor sterols, indicators of cholesterol synthesis...
2. Patients and methods

2.1. Patients

The study group consisted of 16 volunteer males aged 22–79 years (56.2 ± 6.6 years, mean ± SE) and body mass index 24.5 ± 0.5 kg/m². One subject had apo-protein (apo) E phenotype 2/3, nine had apo E 3/3, and six subjects apo E 4/3 phenotype. The different apo E phenotype distribution was not a confounding factor, because the comparisons were made intraindividually. The subjects did not have renal, liver or gastrointestinal diseases or any permanent medication except one subject, who had type 2 diabetes well-controlled with glibenclamide over several years.

The study protocol had been accepted by the Ethics Committee of our Department.

2.2. Methods

2.2.1. Postprandial fat clearance

Each subject participated in two oral fat loads in random order, one with and the other without squalene supplement. After a 12-h fast, the subjects were given a fatty meal containing 90 g of milk fat, 432 mg of cholesterol and 345 000 IU of aqueous vitamin A as a postprandial marker. For the squalene-supplemented test, 500 mg of squalene was added to the fatty meal. The meal was given as a cream-eggshake containing 1200 kcal. After the test meal, the subjects fasted for 9 h, having at 17:00 h their first actual daily meal, which was a low fat, low cholesterol standard hospital meal.

Blood samples were drawn before the test meal and after 3, 4, 6, 9, 12 and 24 h. The blood samples were taken into dark heparin-containing tubes.

2.2.2. Analytical procedures

Commercial kits were used to enzymatically analyze serum total and lipoprotein cholesterol and triglycerides (Boehringer Mannheim Diagnostica, Germany). Chylomicrons were separated from plasma after carefully overlayering with 1.0063 g/ml NaCl salt solution and by ultracentrifugation in a fixed-angle Beckman® rotor for 30 min, followed by density gradient separation of very low density lipoproteins (VLDL, < 1.006 g/ml), intermediate density lipoproteins (IDL, 1.006–1.019 g/ml), low density lipoproteins (LDL, 1.019–1.063 g/ml) and high density lipoproteins (HDL, 1.063–1.210 g/ml) [15]. Postprandial plasma samples of all subjects were separated into chylomicrons, VLDL and VLDL bottom (> 1.006 g/ml), while in six random subjects VLDL bottom was separated into IDL, LDL and HDL.

Serum squalene and noncholesterol sterols Δ⁸-cholestene, desmosterol, lathosterol and plant sterols, campesterol and sitosterol, and cholestanol, a metabolite of cholesterol, were quantitated with gas–liquid chromatography on a 50 m long SE-30 capillary column (Hewlett Packard® Ultra-1) from nonsaponifiable lipids [16,17]. The quantitations were performed from total plasma, chylomicrons, VLDL and VLDL bottom. Lanosterol, the largest methyl sterol peak, and other methyl sterols of Fig. 1 were quantitated in chylomicrons and VLDL.

Apo E phenotypes were determined with isoelectric focusing from plasma [18]. The analysis of retinyl palmitate was carried out with high pressure liquid chromatography [19]. All procedures were completed in subdued light.

2.2.3. Calculations

Postprandial cholesterol, triglyceride and retinyl palmitate concentrations are given as incremental values calculated by subtracting the respective basal fasting value from each postprandial value. Postprandial responses of cholesterol, triglycerides, squalene and retinyl palmitate were also quantitated by calculating the area under the 9-h concentration curve (AUC) for cholesterol and triglycerides and 24-h AUC for squalene and retinyl palmitate for each subject. Areas between the zero level and the respective 9 or 24-h concentration curves were also calculated (AUIC).

Serum cholesterol precursor sterols are given as propor-
3. Results

Baseline serum lipids were as follows: serum cholesterol 5.18 ± 0.23 mmol/l, LDL cholesterol 3.05 ± 0.19 mmol/l, HDL cholesterol 1.26 ± 0.07 mmol/l and serum triglycerides 1.18 ± 0.09 mmol/l. Fasting serum squalene concentration was 163 ± 11 µg/dl and was distributed in lipoproteins as follows: 9.5 ± 2.0% in chylomicrons, 11.8 ± 1.9% in VLDL, 8.6 ± 1.2% in IDL, 39.8 ± 4.2% in LDL and 30.3 ± 2.2% in HDL. On the contrary, two-thirds of postprandial squalene was carried in triglyceride-rich lipoproteins of density < 1.006 g/ml (Fig. 2).

After supplementation, postprandial squalene AUC (calculated from values in Fig. 2) was significantly increased by 92% to 868% in chylomicrons, VLDL and VLDL bottom compared with the fat load without squalene supplementation ($P < 0.05$ or less for all, Table 1, Fig. 2). In LDL and HDL, assayed in six random subjects, squalene only tended to increase. Postprandial peak times in chylomicrons and VLDL were 4.3 ± 0.3 and 4.8 ± 0.6 h for triglycerides, 8.1 ± 0.8 and 8.8 ± 0.7 h for retinyl palmitate ($P < 0.05$ from triglycerides), and 8.5 ± 0.7 h and 9.3 ± 0.6 h for squalene ($P < 0.05$ from triglycerides), respectively. The differences of the peak times were not significant between chylomicrons and VLDL.

Addition of the single squalene dose to the test meal had no effect on the postprandial cholesterol, triglyceride and retinyl palmitate values in chylomicrons (Fig. 3), VLDL (Fig. 4) and VLDL bottom (data not shown).

Cholesterol synthesis expressed by lathosterol/campesterol ratios in chylomicrons, VLDL and VLDL bottom were diminished after the fat load without squalene up to 12 h, after which the ratio returned to the baseline at 24 h or significantly above it in VLDL bottom (Fig. 5). $\Delta^8$-cholestenol/campesterol ratio was

![Graphs showing lipoprotein distributions and peak times](image)

**Table 1**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>AUC 1</th>
<th>AUC 2</th>
<th>Difference</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2502 ± 206</td>
<td>8095 ± 578*</td>
<td>5593 ± 599*</td>
<td>247 ± 32*</td>
</tr>
<tr>
<td>Chylomicon</td>
<td>291 ± 31</td>
<td>2562 ± 318*</td>
<td>2271 ± 317*</td>
<td>868 ± 113*</td>
</tr>
<tr>
<td>VLDL</td>
<td>453 ± 57</td>
<td>2383 ± 293*</td>
<td>1930 ± 288*</td>
<td>530 ± 103*</td>
</tr>
<tr>
<td>VLDL bottom</td>
<td>1819 ± 199</td>
<td>3150 ± 182*</td>
<td>1332 ± 215*</td>
<td>92 ± 17*</td>
</tr>
</tbody>
</table>

* Mean ± SE. AUC, area under curve; µg/dl per h.  
* $P < 0.05$. 

---

**Fig. 2.** Postprandial squalene in different lipoprotein fractions. Mean values. ○, squalene in test meal; •, no squalene in test meal. (N = 16 in chylomicrons and VLDL; N = 6 in IDL, LDL and HDL). *$P < 0.05$ from non-squalene values, analysis of variance and covariance with repeated measures, $t$-test.

---

2.2.4. Statistical significance

Statistical significances were tested with analysis of variance and covariance for repeated measures within and between the two experiments, and with Student’s two-sided $t$-test and paired $t$-test. Logarithmic transformations were used when the distributions were skewed. A $P$ value of $<0.05$ was considered statistically significant.
not changed consistently, while desmosterol/campesterol curve resembled that of lathosterol/campesterol (data not shown).

Lathosterol/campesterol curves after squalene supplementation differed significantly in chylomicrons, VLDL and VLDL bottom from the respective non-supplemented curves ($P = 0.0000–0.0005$, analysis of variance and covariance for repeated measures). Squalene addition caused an increase in lathosterol/campesterol ratio starting after 6 h in chylomicrons and VLDL, and reached significantly higher values at 12 h than the non-squalene results (Fig. 5). Squalene-supplemented values started to diverge after 6 h from the non-squalene values in VLDL bottom and were significantly higher at 9 and 12 h (Fig. 5). The results obtained using desmosterol instead of lathosterol were identical in VLDL whereas in the other lipoprotein fractions the curves did not differ significantly with and without squalene. Postprandial $\Delta^8$-cholestenol was not influenced by squalene supplementation. In addition, VLDL but not chylomicron lanosterol/campesterol ratio at 12 h was $0.069 \pm 0.012$ without and $0.141 \pm 0.028$ with squalene addition ($P < 0.05$), while the changes of other methyl sterols remained inconsistent (data not shown). Using sitosterol instead of campesterol in calculations gave equal results to campesterol in VLDL and VLDL bottom. Postprandial serum plant sterol levels remained unchanged after the test meal.

4. Discussion

This study shows for the first time that a single 500 mg dose of orally administered squalene increases cholesterol synthesis in humans within 9–24 h, measured by increased lanosterol or lathosterol/plant sterol ratios. Lathosterol is the largest cholesterol precursor sterol fraction in serum and its quantitation is simple...
Increases in precursor sterol/plant sterol ratios in chylomicrons, which are secreted by the intestine, suggest that squalene is partly converted to cholesterol in intestinal mucosa, which has a significant capacity to synthesize cholesterol [24]. It is suggested that the newly absorbed squalene was converted to precursor sterols and further to cholesterol also in the liver due to the high postabsorptive squalene concentrations in VLDL bottom. It is, however, not possible to distinguish specifically proportions of intestinal and hepatic cholesterol synthesis by precursor sterol/plant sterol ratios.

Postprandial increase in lipoprotein squalene has earlier been described 3-4 hours after a fat meal containing squalene [25]. More recently, postprandial squalene in healthy subjects has been shown to reach maximum concentration much later, at 8–10 h after oral squalene administration [12,13], which is in agreement with the present study and very similar to the respective peak times of retinyl palmitate. Thus, it could be assumed that the effect of squalene on cholesterol metabolism cannot be seen earlier than 6–9 h after oral administration. In the present study, in concordance with earlier observations, about one-third of fasting squalene is transported in triglyceride-rich lipoproteins (density < 1.019 g/ml), whereas of the postprandial squalene about two-thirds can be recovered from these lipoprotein fractions [12,13]. Intestinal absorption of squalene has been estimated to be 60% [10]. Squalene could theoretically interfere with the absorption of other lipid-soluble components which are absorbed in micelles [26]. The present data, however, suggest that 500 mg of squalene given with cream-eggshake meal does not interfere with postprandial cholesterol, triglyceride or retinyl palmitate concentrations.

Diurnal variation of cholesterol synthesis has been shown in experimental animals [27–29] and in human subjects [30]. Decrease of cholesterol synthesis is revealed by lowered precursor sterols in serum at noon and afternoon hours, followed by their rise towards the evening reaching the maximum at night at 02:00–04:00 h [30]. Similarly, in this study cholesterol synthesis was decreased, indicated by lowered lathosterol/campesterol ratio during the day time, and it did not reach baseline levels until the next morning. However, when squalene is added to the test meal, cholesterol synthesis is upregulated at 9–12 h, indicated by increased lathosterol/campesterol ratio in chylomicrons, VLDL and VLDL bottom.

This study helps us to understand mechanisms of the effect of cholesterol precursor feeding, squalene in the present case, on serum cholesterol levels. Previous observations on daily squalene consumption have revealed inconsistent results. Chronic intake of squalene, (1 g/day) mimicking a diet rich in squalene, led to increased cholesterol precursor sterols and raised cholesterol concentrations, but a smaller dose of squalene (0.5 g/day)
normalized the situation [11]. On the other hand, squalene consumption of 0.86 g/day has been found even to reduce serum cholesterol [14]. This study showed that a single dose of 0.5 g of squalene was enough to increase cholesterol synthesis indicated by precursor sterols from 9 to 12 h after ingestion. The previous observation that daily consumption of 0.5 g of squalene does not increase serum cholesterol levels [11] and the present observation that the same amount as a single dose acutely increases cholesterol synthesis needs explanation. Possible compensatory mechanisms to prevent the increase of serum cholesterol during a moderate long-term squalene consumption are reduced HMG-CoA reductase activity and increased fecal elimination of cholesterol as cholesterol itself and bile acids [10].

Increased serum squalene concentrations have recently been described in postmenopausal women with coronary artery disease [31]. There is, however, a conflict considering the lack of association between high squalene intake and coronary artery disease in Mediterranean populations consuming lots of olive oil [32]. The range of squalene in olive oil is wide (1.5–9.5 mg squalene in 1 g oil) [1,11]. Daily doses that elevate serum cholesterol levels, i.e. 1 g of squalene in 1 g oil) [1,11]. Daily doses that elevate serum cholesterol levels, i.e. 1 g/day, can be reached in individuals consuming large amounts (> 100 g/day) of olive oil. On the other hand, squalene has been reported to exhibit cellular radioprotective effects probably due to antioxidant properties [33]. Thus, the role of squalene in relation to coronary artery disease is complicated, and remains to be further studied.

Acknowledgements

This study was supported by grants from the Research Institute of Orion Corporation, Clinical Research Institute of the Helsinki University Central Hospital, the Helsinki University Central Hospital and the Finnish Academy, Council of Medical Sciences. The excellent technical assistance of Ms Leena Kaipiainen, Pia Hoffström, Orvokki Ahlroos, Ritva Nissilä and Mr Antti Laine, M.Sc., is greatly acknowledged.

References