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Postprandial behavior of plasma squalene and non-cholesterol sterols in men with varying cholesterol absorption

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Abstract

Background: The purpose of this study was to investigate, whether low vs. high absorption of cholesterol affects the postprandial lipid clearance (squalene as the surrogate marker) and postprandial cholesterol metabolism evaluated with plasma levels of cholesterol absorption (cholestanol and plant sterols) and synthesis markers (desmosterol and lathosterol).
Methods: Fifteen normo- or mildly hypercholesterolemic men were divided into low or high cholesterol absorbers on the basis of plasma cholestanol to cholesterol ratio and they volunteered to an oral fat load test containing fat 35 g/m^2 body surface.
Results: Plasma squalene to cholesterol ratio did not differ between the groups throughout the postprandial follow-up of 8 h. The level differences in the plasma absorption and synthesis markers seen at baseline remained between the groups, so that in high absorbers the absorption markers remained high and synthesis markers low throughout the postprandial follow-up. The postprandial response curves of desmosterol ($p<0.05$) and lathosterol ($p=0.052$) to cholestanol decreased linearly in the low, but not in the high absorbers.
Conclusions: Low vs. high absorption of cholesterol does not affect the first 8-h postprandial lipid clearance. The metabolic profile of cholesterol is maintained postprandially. The postprandial decrease in cholesterol synthesis differs in low vs. high absorbers especially through the desmosterol pathway.

Keywords: Postprandium; Squalene; Desmosterol; Cholestanol; Cholesterol absorption; Cholesterol synthesis

1. Introduction

In humans, cholesterol metabolism is not homogenous, but, instead varies between individuals. Based on cholesterol homeostasis, subjects with high absorption have low de novo synthesis of cholesterol, and those with low absorption have high synthesis of cholesterol. The profile of cholesterol metabolism varies in some diseases. For instance, in post-menopausal women with coronary artery disease cholesterol synthesis was reduced compared with controls [1]. The coronary women had also impaired postprandial lipid clearance [2]. Within coronary subjects, the profile of cholesterol metabolism determined the risk of recurrences of major coronary events during simvastatin treatment [3] such that the risk was reduced in subjects with high, but not with low cholesterol synthesis in spite of similar serum lipid levels. Ballantyne et al. [4] showed that the untreated coronary subjects with poor prognosis had, in addition to elevated LDL cholesterol concentration, elevated serum triglycerides and low HDL cholesterol associated with other characteristics of the metabolic syndrome. These subjects resembled those with high cholesterol synthesis and low absorption in our 4S subgroup [3,5]. These results suggested that in coronary women per se cholesterol synthesis is low, absorption high and postprandial lipid clearance delayed, but within coronary subjects, those with high cholesterol synthesis and characteristics of metabolic syndrome have worse prognosis without treatment, whereas

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inhibition of cholesterol synthesis with statins is beneficial especially in subjects with high cholesterol synthesis. Second, cholesterol synthesis is high and absorption efficiency low in obesity [6], metabolic syndrome (MBO) [7,8], insulin resistance [9] and type 2 diabetes [10], in which also postprandial lipid clearance is delayed. The question now arises, whether the different profiles of cholesterol metabolism affect postprandial lipid clearance or postprandial cholesterol metabolism. The lack of difference in postprandial triglycerides between low and high absorbers in our recent study [11] does not exclude the possibility that postprandial fat and cholesterol metabolism could differ between these groups; in spite of similar postprandial triglyceride levels the lipid clearance assayed in detail was different [2]. Therefore, we evaluated, whether the postprandial level of serum squalene, a marker of postprandial lipid clearance [2,12] and the markers of cholesterol absorption and synthesis [13,14] differ in subjects with low and high cholesterol absorption. The latter was defined with baseline serum cholestanol to cholesterol ratio, a surrogate marker of cholesterol absorption [13].

2. Subjects and methods

2.1. Subjects

Altogether 15 normo- or mildly hypercholesterolemic men aged 29–71 years (52±3 years, mean±S.E.M.) were recruited to this investigation from our former study populations at the Department of Clinical Nutrition. Their body mass index (BMI) was 27.7±0.7 kg/m^2 and serum total cholesterol and triglyceride concentrations were 5.42±0.23 mmol/l and 1.06 ±0.08 mmol/l, respectively. None of the subjects had liver, kidney, thyroid, or coronary artery disease, or diabetes mellitus or gastrointestinal diseases. One subject used calcium channel blockers, two beta blocking agents, and two ACE inhibitors for hypertension. None of the subjects had lipid-lowering therapy or plant stanol or -sterol products. Two were smokers. The subjects were requested to maintain their medication, weight, diet, possible alcohol consumption, smoking habits and physical activity constant before the study. One man was excluded from the statistical analysis due to exceptionally high postprandial triglyceride response (area under the response curve differed over 4 standard deviations from the mean of other subjects).

The subjects were divided into median groups by baseline plasma cholestanol to cholesterol ratio (131×10^2 mmol/mol of cholesterol), so that the median illustrated low (≤131) and high (>131) absorption efficiency of cholesterol. Baseline characteristics of the subjects are presented in Table 1.

The subjects gave their informed consent for the study, and the study protocol was approved by the Ethics Committee of the University of Kuopio.

2.2. Dietary records

The subjects kept 7-day food record (5 weekdays and 2 weekend days) just before the study. They recorded their food consumption using portion size booklet with photos to estimate the portion size [15]. Mean intake of nutrients were calculated only from the 6 days, because the fasting began in the evening of the last recording day. The nutrients in the food records were calculated using the MicroNutrica® dietary analysis program (version 2.5, Finnish Social Insurance Institute, Turku, Finland). The food composition database is based on analyses of the Finnish food and international food composition tables [16].

Table 1
Baseline characteristics of the subjects divided to low and high absorbers by baseline plasma cholestanol to cholesterol ratio

	Low absorbers (n=7)	High absorbers (n=7)	p [a]
Age (years)	51.6±6.2	52.3±4.0	0.925
Body mass index (kg/m^2)	28.0±1.0	26.6±0.9	0.306
Waist circumference (cm)	99.8±2.5	99.9±1.6	0.974
Smokers (n)	1	1	1
Users of beta-blocker (n)	1	1	1
Users of calcium channel blocker (n)	0	1	1
Users of renin–angiotensin system affecting medication (n)	1	1	1
Plasma glucose (mmol/l)	6.19±0.34	5.87±0.17	0.496
Serum insulin (mU/l)	9.61±1.03	7.86±0.64	0.172
Homeostasis model assessment (HOMA)	2.65±0.33	2.05±0.19	0.142
Serum cholesterol (mmol/l)	4.79±0.29	6.00±.021	0.006
VLDL cholesterol (mmol/l)	0.30±0.05	0.17±0.02	0.030
LDL cholesterol (mmol/l)	3.33±0.29	4.06±0.33	0.124
HDL cholesterol (mmol/l)	1.07±0.08	1.63±0.21	0.030
Serum triglycerides (mmol/l)	1.13±0.12	0.92±0.09	0.170
VLDL triglycerides (mmol/l)	0.59±0.08	0.39±0.07	0.078
LDL triglycerides (mmol/l)	0.25±0.02	0.27±0.04	0.574
HDL triglycerides (mmol/l)	0.15±0.01	0.14±0.01	0.606

Low absorbers: plasma cholestanol to cholesterol ratio ≤131×10^2 mmol/mol of cholesterol. High absorbers: plasma cholestanol to cholesterol ratio >131×10^2 mmol/mol of cholesterol.

[a] Indicates the significance between the low and the high absorbers analyzed with Student's t-test test or Fisher exact test (category variables).

2.3. Oral fat-loading test

The oral fat-loading test started at 7:30–8:00 am after a 12 h fast. Subjects were advised not to drink alcohol and to avoid strenuous exercise for 3 days before the test. After collecting fasting blood samples, subjects consumed a drink containing a mixture of milk and vegetable fat-based creams, fish oil (9.3 g/m^2 body surface), yolk (150 mg/m^2 body surface), and squalene (250 mg/m^2 body surface) as a postprandial marker. The cream mixture contained in the ratio of 1:1 cream (38% fat) (Valio, Finland) and vegetable fat-based cream (20% fat) (Unilever Finland Oy). The amount of total fat load was 35 g/m^2 body surface and that of cholesterol 234 mg/m^2 body surface. After the test meal, postprandial blood samples were collected at 15 (only glucose and insulin), 30, 45 (only glucose and insulin) and 60 min, and 2, 3, 4, 6 and 8 h.