Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes[1]

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Abstract The aim of the present study was, first, to investigate whether cholesterol (C) absorption, enhanced by cholesterol feeding, was related to synthesis of cholesterol, serum level of low density lipoprotein (LDL)-C, and receptor activity for LDL apolipoprotein (apo) B in healthy men. Secondly, we were interested in whether apolipoprotein E (apoE) phenotypes contributed to cholesterol and LDL apoB metabolism under these conditions. We studied 29 home-living men aged 55 ± 1 (mean ± SE) years on a low-fat, low cholesterol (208 ± 13 mg/day) diet followed by a low-fat high cholesterol (878 ± 38 mg/day) diet during 5 weeks. Cholesterol feeding increased total cholesterol, LDL-C, high density lipoprotein (HDL)-C, and LDL apoB levels from 10% to 13% ($P < 0.05$) and bile acid production and cholesterol turnover by 16% ($P < 0.05$), decreased the fractional catabolic rate (FCR) for LDL apoB by 10% ($P < 0.05$) and cholesterol absorption efficiency by 8% ($P < 0.05$), while cholesterol synthesis only tended to decrease. During the cholesterol feeding, LDL-C was positively related to apoB production rate and cholesterol absorption efficiency ($P < 0.05$), and negatively related to bile acid and cholesterol synthesis ($P < 0.05$) and FCR for LDL apoB, which, in turn, was negatively related to cholesterol absorption efficiency and positively to bile acid synthesis. ApoE phenotype was positively related to TC, LDL-C, and LDL apoB levels and negatively to FCR for LDL apoB. The increase of the LDL-C level by the high cholesterol intake was positively correlated with LDL-C on high cholesterol diet and apoE phenotypes, so that the increase was 7% in apoE2 (ns), 11% in apoE3 ($P < 0.05$), and 18% in apoE4 ($P < 0.05$); the increase of bile acid synthesis was significant only in subjects with apoE2. Moreover, the increase of LDL-C was positively related to the absolute amount of dietary cholesterol absorbed and negatively to FCR for LDL apoB. The findings suggest that the higher the LDL-C level, the higher is the absorption efficiency of cholesterol and production of LDL apoB, and the lower is the removal of LDL apoB and synthesis of both bile acids and cholesterol, and the more frequently the subjects had $\epsilon 4$ allele. The nonresponsiveness to dietary cholesterol was dependent on low LDL-C level, apoE2 phenotype, and effective bile acid synthesis. Subjects with $\epsilon 2$ allele were nonresponders, while subjects with $\epsilon 3$ or $\epsilon 4$ alleles were sensitive to augmented dietary cholesterol.—**Gylling, H., and T. A. Miettinen.** Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. *J. Lipid Res.* 1992. **33:** 1361–1371.

Supplementary key words bile acids • kinetics of LDL apolipoprotein B • receptor activity

High cholesterol intake generally increases the serum levels of total (TC) and low density lipoprotein cholesterol (LDL-C). If dietary fat is kept constant, the increase in serum LDL-C after physiologically augmented cholesterol intake varies from 4 to 58% (1–9). Up to a moderate daily cholesterol intake of 400 mg, serum TC will be linearly increased (10). During high cholesterol intake, several feedback responses have been documented. Cholesterol synthesis is frequently (1, 7, 11–14) but not consistently (15, 16) down-regulated, while the other documented responses are even more controversial. Cholesterol absorption varies from depressed (7, 16) to unchanged (13) or to increased (1) values. Bile acid synthesis varies also from unchanged or slightly decreased (11–13) to an enhanced rate (1, 15, 16). Resecretion of absorbed cholesterol may be increased (1, 11–13, 17). LDL apolipoprotein B (apoB) catabolism is sluggish (4) and LDL apoB receptor activity is down-regulated in monocytes (2, 5), although unchanged in fibroblasts (3). LDL apoB production rate has been documented in only one study where it was increased (4).

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; C, cholesterol; TC, total cholesterol; FCR, fractional catabolic rate; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein.

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Cholesterol challenge does not invariably result in serum LDL-C elevation (7, 8, 12, 15, 18, 19). Again, the metabolic responses vary in different studies. In nonresponders with practically unaltered serum TC and LDL-C, an effective suppression of hepatic cholesterol synthesis (7, 12, 20), an increment in bile acid synthesis (15, 16), and/or a decrement in intestinal cholesterol absorption efficiency (7, 15, 16) have been reported. Second, apolipoprotein E (apoE) phenotype is deeply involved in cholesterol homeostasis. Subjects with apoE4 phenotype have an elevated serum LDL-C level, enhanced absorption efficiency of cholesterol, and lower cholesterol synthesis rate than subjects with $\epsilon 2$ allele (21–23). ApoE phenotype is associated (24, 25), although not consistently (8, 26, 27), with alterations in serum TC and LDL-C levels due to modifications in dietary cholesterol.

The aim of the present study was, first, to investigate the effects of a high cholesterol intake on serum lipids, lipoproteins, cholesterol absorption, elimination, and synthesis, and LDL apoB kinetics in healthy, home-living subjects. Second, the responsiveness of serum lipids to dietary cholesterol was evaluated relating different apoE phenotypes to the variables of cholesterol and LDL apoB metabolism during different cholesterol intakes. The present study consists of a part of a large intervention, of which the effects of dietary fat and cholesterol reduction from baseline home diet will be published separately (28).

STUDY POPULATION AND METHODS

Study population

The study group consisted of 29 healthy male subjects aged 55 ± 1 (mean ± SE) years with different apoE phenotypes (**Table 1**). The participants were volunteers from a random male population of about 350 subjects, augmented by occasionally discovered individuals with the $\epsilon 2$ allele. As compared with the normal population, the number of subjects with the $\epsilon 4$ allele was also increased so as to get different apoE phenotype groups large enough for statistical treatment. The apoE2 group included two subjects with apoE 2/2, five with apoE 3/2, and one with apoE 4/2 phenotype. The apoE3 group included nine subjects with apoE 3/3 phenotype, and the apoE4 group included eleven subjects with apoE 4/3 and one with apoE 4/4 phenotype. Correlations of the lipid and metabolic data with apoE phenotypes were carried out to the apoE subscripts as follows: apoE 2/2 = 1, apoE 3/2 = 2, apoE 4/2 = 3, apoE 3/3 = 4, apoE 4/3 = 5, and apoE 4/4 = 6.

The subjects were given a thorough medical examination including laboratory tests and ECG at rest. None of them had any major medical problems including liver, thyroid, or renal diseases, diabetes or endocrinologic or malignant disease. One subject had coronary artery disease and five had elevated blood pressure. Four subjects were on beta-blocking agents and one on a calcium channel-blocking agent. None had had lipid-lowering medication. All subjects gave an informed consent, and the study protocol had been accepted by the Ethics Committee of our hospital.

Experimental design

The study lasted for 11 weeks. The subjects were studied at the outpatient ward every 2 weeks. At baseline on home diet the mean cholesterol intake was 574 ± 23 (mean ± SE) mg/day. After entering the study, a low-cholesterol, low-fat diet with 24% of calories as fat mimicking the American Heart Association Diet Two was started for 6 weeks (Table 1). After this period cholesterol was added to the diet for 5 weeks as three egg yolks/day and the subjects were advised to reduce a respective amount of daily dietary fat. Dietary counseling was carried out as oral and written personal instructions from our dietician at the start of the study and twice during each period.

TABLE 1. Age, height, body weight, and dietary parameters during low (L) and high (H) cholesterol diets

Variables	Diet	All n = 29	ApoE2 n = 8	ApoE3 n = 9	ApoE4 n = 12
Age (yr)		54 ± 1	49 ± 3	55 ± 2	55 ± 2
Height (m)		1.75 ± 0.01	1.76 ± 0.02	1.75 ± 0.02	1.75 ± 0.02
Weight (kg)	L	81 ± 2	83 ± 4	82 ± 4	79 ± 4
	H	80 ± 2[a]	82 ± 4[a]	82 ± 4	78 ± 4
Cholesterol intake (mg/day)	L	208 ± 13	224 ± 15	216 ± 33	193 ± 19
	H	878 ± 38[a]	965 ± 67[a]	875 ± 86[a]	823 ± 49[a]
Fat intake (g/day)	L	53 ± 3	52 ± 6	59 ± 6	49 ± 6
	H	69 ± 3[a]	68 ± 3[a]	77 ± 7[a]	64 ± 6[a]
P/S	L	0.72 ± 0.05	0.66 ± 0.04	0.84 ± 0.09	0.66 ± 0.08
	H	0.63 ± 0.04	0.68 ± 0.06	0.67 ± 0.08	0.57 ± 0.06

Values given as means ± SE. ApoE2 denotes phenotypes apoE 2/2, 3/2, or 4/2. ApoE3 denotes phenotype apoE 3/3. ApoE4 denotes phenotypes apoE 4/3 and apoE 4/4. P/S = polyunsaturated/saturated fatty acid ratio in dietary fat.
[a] $P < 0.05$ or less compared to low cholesterol diet.

Blood samples were drawn after an overnight fast four times during each dietary period for lipid and lipoprotein analyses. Cholesterol absorption, sterol balance, and LDL apoB kinetics were studied at the end of each period.

Analysis of serum lipids, lipoproteins, and apoproteins

Commercial kits were used to analyze serum cholesterol and triglycerides (Boehringer Mannheim Diagnostica, Germany), and apolipoproteins (apo) A-I and B (Orion Diagnostica, Finland). ApoB was analyzed also in very low (VLDL) and intermediate density lipoproteins (IDL) and LDL. In the kinetic studies, LDL apoB was analyzed after isopropyl alcohol precipitation (29, 30), and the protein mass was measured by the procedure of Lowry et al. (31). Lipoproteins were separated by ultracentrifugation in density classes as described in Manual of Laboratory Operations of Lipid Research Clinics Program (32). ApoE phenotyping was performed by isoelectric focusing from serum (33).

For LDL apoB kinetic studies, LDL was separated by ultracentrifugation from density 1.019–1.063 g/ml. LDL apoB was iodinated with ^{125}I by a modification (34) of the iodine monochloride method (35). Free iodine was separated by chromatography and extensive dialyses. A sample of LDL (20–30 μCi) was sterilized by membrane filtration (Millipore, 0.22 μm) and injected intravenously. Three days before injection the subjects started to take oral potassium iodide. Blood samples of 10 ml were collected after injection over a 14-day period and counted. The die-away curves were constructed in whole plasma for ^{125}I, and the curves were analyzed according to the two-pool model of Matthews (36). Plasma volume was estimated as 4.5% of body weight.

Cholesterol absorption, fecal sterols, and cholesterol synthesis

Cholesterol absorption was measured with the oral double-isotope feeding method (37) and cholesterol and bile acid synthesis and fecal sterol excretion were measured with the sterol balance technique. Therefore, the subjects consumed three capsules a day for 7 days, each containing [^{14}C]cholesterol and [^{3}H]sitosterol and 200 mg chromic oxide (Cr_2O_3) with each major meal to correct for fecal flow. After a stabilization period of 4 days, a 3-day stool collection was carried out and analyzed for radioactivities, fecal sterols (38–40), and Cr_2O_3 (41). Recovery of [^{3}H]sitosterol was 100% in feces and, in separate analyses, the recovery values of the first and third collection days were similar. During the 7-day period, a diet record was also made for calculation of dietary cholesterol and fat intake (42). The calculations were: cholesterol synthesis = total fecal steroids (neutral sterols + bile acids) of cholesterol origin – dietary cholesterol; dietary cholesterol absorbed = cholesterol absorption efficiency × dietary cholesterol; and cholesterol turnover = fecal neutral sterols – (1 – fractional cholesterol absorption) × dietary cholesterol + fecal bile acids.

Statistical analyses were performed with Student's two-sided *t*-test, paired *t*-test, analysis of variance (ANOVA), and Pearson's correlation. A *P*-value less than 0.05 was considered significant.

RESULTS

Whole study group

Dietary changes. Subjects on the high cholesterol diet lost approximately 1 kg of their body weight (Table 1) because the caloric intake was significantly decreased by 6%. Fat intake increased from 53 g/day to 69 g/day corresponding to 30% of calories as fat. The quality of daily fat remained constant and the P/S ratio was essentially unchanged. The increased fat intake and the decrease in body weight were similar in all apoE phenotypes. Dietary cholesterol intake increased from 208 to 878 mg/day.

Serum lipids and LDL apoB kinetics. Serum levels of TC, LDL-C, and LDL apoB were increased significantly by 10 ± 2% (mean ± SE), 13 ± 2%, and 9 ± 2%, respectively (**Table 2** and **Table 3**). HDL-C and apoA-I levels were increased significantly by 13 ± 2% and 9 ± 2% (Table 2). Other serum lipoprotein and triglyceride levels were unchanged. The particle compositions of VLDL, IDL, or LDL described as the cholesterol/apoB ratio, were not consistently changed.

The fractional catabolic rate (FCR) for LDL apoB was significantly decreased by 10 ± 2% (Table 3 and **Table 4**), while LDL apoB production was unchanged.

Cholesterol absorption and metabolism. Cholesterol absorption efficiency was slightly but significantly decreased by 8 ± 3% in the whole study group during the high cholesterol intake (Table 4 and **Table 5**). The absolute amount of dietary cholesterol absorbed was increased fourfold from 1.03 ± 0.08 mg/kg per day to 4.0 ± 0.3 mg/kg per day.

Fecal total and neutral sterol (Table 5) outputs were significantly increased by 59 ± 7% and 83 ± 10%, reflecting mostly the increased amount of dietary unabsorbed cholesterol. Fecal bile acids (Tables 4 and 5) and total endogenous steroid output, i.e., cholesterol turnover rate (12.1 ± 0.5 vs. 14.1 ± 0.8 mg/kg per day), were also significantly increased. Cholesterol synthesis decreased insignificantly by 7%. Consequently, increased cholesterol intake slightly lowered cholesterol absorption efficiency, increased absolute absorption of dietary cholesterol, bile acid synthesis, and cholesterol turnover rate, but only tended to slow down cholesterol synthesis, and reduced removal of LDL apoB so that the LDL-C level was increased in serum.

Correlations during high cholesterol intake. Dietary fat, unrelated to the absorption efficiency of cholesterol, was as-

TABLE 2. Serum lipids, lipoproteins, and apoproteins during low (L) and high (H) cholesterol and low fat diets

Fraction	Diet	All n = 29	$ApoE_2$ Group n = 8	$ApoE_3$ Group n = 9	$ApoE_4$ Group n = 12
Total cholesterol, mg/dl	L	194.6 ± 6.8	166.8 ± 11.5	212.4 ± 10.9[a]	199.8 ± 9.5[a]
	H	214.8 ± 7.8[b]	176.4 ± 11.7	232.3 ± 9.6[a,b]	227.3 ± 11.9[a,b]
Total triglycerides, mg/dl	L	142.1 ± 10.2	143.2 ± 20.8	125.4 ± 18.1	153.8 ± 15.7
	H	138.7 ± 11.2	150.1 ± 21.3	124.2 ± 24.4	142.0 ± 15.1
VLDL cholesterol, mg/dl	L	20.4 ± 1.9	24.2 ± 5.1	17.3 ± 3.3	20.2 ± 2.2
	H	19.1 ± 2.4	24.0 ± 6.2	16.2 ± 4.3	17.9 ± 2.5
VLDL triglycerides, mg/dl	L	84.2 ± 8.2	89.3 ± 17.2	69.8 ± 13.1	91.6 ± 13.1
	H	79.5 ± 8.6	93.8 ± 18.4	67.0 ± 16.8	79.3 ± 11.5
VLDL apoB, mg/dl	L	19.4 ± 2.1	21.3 ± 4.6	16.7 ± 3.5	20.3 ± 3.1
	H	18.3 ± 2.4	22.9 ± 5.9	15.9 ± 4.7	17.0 ± 2.6
IDL cholesterol, mg/dl	L	7.9 ± 0.6	8.8 ± 1.1	8.7 ± 1.0	6.8 ± 0.9
	H	7.9 ± 0.7	8.1 ± 1.5	8.3 ± 1.2	7.5 ± 1.0
IDL triglycerides, mg/dl	L	5.9 ± 0.3	6.3 ± 0.7	6.2 ± 0.6	5.3 ± 0.4
	H	6.1 ± 0.4	6.1 ± 0.8	6.1 ± 0.9	6.1 ± 0.5
IDL apoB, mg/dl	L	3.5 ± 0.2	3.9 ± 0.4	3.8 ± 0.4	3.1 ± 0.3
	H	3.6 ± 0.3	3.7 ± 0.6	3.8 ± 0.5	3.4 ± 0.4
LDL cholesterol, mg/dl	L	103.6 ± 6.0	71.7 ± 8.4	121.0 ± 10.6[a]	111.8 ± 6.7[a]
	H	117.4 ± 7.2[b]	76.7 ± 9.9	134.5 ± 9.0[a,b]	131.7 ± 9.5[a,b]
LDL triglycerides, mg/dl	L	19.7 ± 1.2	15.4 ± 1.8	21.3 ± 1.7[a]	21.5 ± 2.1[a]
	H	20.9 ± 1.4	15.5 ± 1.5	22.8 ± 2.5[a]	23.0 ± 2.4[a]
LDL apoB, mg/dl	L	45.8 ± 2.8	32.0 ± 3.7	53.2 ± 4.7[a]	49.4 ± 3.4[a]
	H	50.0 ± 3.1[b]	31.8 ± 4.4	58.9 ± 4.0[a,b]	55.4 ± 3.7[a,b]
LDL cholesterol/apoB	L	1.88 ± 0.02	1.87 ± 0.04	1.91 ± 0.05	1.87 ± 0.03
	H	1.93 ± 0.02	1.87 ± 0.02	1.90 ± 0.06	1.99 ± 0.02[a,b]
HDL cholesterol, mg/dl	L	43.6 ± 1.7	43.7 ± 2.6	46.3 ± 1.9	41.4 ± 3.4
	H	49.2 ± 1.9[b]	48.0 ± 3.1[b]	51.0 ± 2.5[b]	48.7 ± 3.8[b]
HDL triglycerides, mg/dl	L	13.6 ± 0.6	15.0 ± 1.1	13.0 ± 1.5	13.1 ± 0.8
	H	14.7 ± 0.7	15.6 ± 1.0	14.4 ± 2.0	14.2 ± 0.6
ApoA-I, mg/dl	L	101.1 ± 3.1	103.8 ± 4.8	105.2 ± 5.4	96.3 ± 5.5
	H	109.8 ± 3.0[b]	110.2 ± 5.5[b]	112.9 ± 4.1[b]	107.3 ± 5.6[b]

Values given as mean ± SE.
[a]Significantly different from apoE2.
[b]Significantly different from low cholesterol intake.

sociated with the absolute amount of dietary cholesterol absorbed ($r = 0.588$). In addition, cholesterol absorption efficiency was inversely correlated with FCR for LDL apoB ($r = -0.476$, **Fig. 1**).

The change in LDL-C was poorly related to cholesterol absorption, elimination, and synthesis, but was positively correlated with apoE subscript (Fig. 1), the LDL-C level ($r = 0.459$), the absolute amount of dietary cholesterol absorbed ($r = 0.437$), and inversely with FCR for LDL apoB ($r = -0.496$).

ApoE phenotypes

The added cholesterol intake increased TC, LDL-C, and LDL apoB values in apoE3 and E4 but not in E2 subjects (Tables 2-4). Thus, LDL-C increased nonsignificantly by 7% in the subjects with the $\epsilon 2$ allele, significantly by +11% ($P < 0.05$) in those with $\epsilon 3$, and by +18% ($P < 0.05$) in those with $\epsilon 4$ alleles. VLDL and IDL lipid levels were similar in apoE groups, but LDL triglycerides were low in apoE2 during both diets. HDL-C increased similarly in different apoE groups during the high cholesterol intake. The latter diet increased the LDL-C/apoB ratio in apoE4, suggesting an enrichment of cholesterol content in the lipoprotein particle of this specific apoE group (Table 2).

The subjects with $\epsilon 2$ allele had the fastest catabolism and tended to have the lowest production rate of LDL apoB (Table 3). FCR for LDL apoB was, in fact, negatively related to the apoE subscript ($r = -0.777$, Fig. 1) and positively to bile acid synthesis ($r = 0.490$), but not cholesterol synthesis during the high cholesterol intake.

Cholesterol absorption efficiency was lowest in the apoE2 subjects although the difference was significant only during the low cholesterol intake (Table 5). During the latter diet the only metabolic alteration differing between the apoE phenotypes was a significantly increased bile acid synthesis in the apoE2 subjects (Tables 4 and 5). The increase in dietary cholesterol absorption was highest (+3.2 ± 0.2 mg/kg per day) and the decrease in cholesterol synthesis was lowest (−0.2 ± 1.3 mg/kg per day) in

TABLE 3. Serum total and lipoprotein cholesterol and LDL apoB kinetics during low and high cholesterol intake

Subjects, ApoE Phenotype	Dietary cholesterol		Cholesterol: Total		Cholesterol: LDL		LDL ApoB		FCR LDL ApoB		PR LDL apoB	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	mg/kg/day		*mg/dl*				*mg/dl*		*pools/day*		*mg/kg/day*	
E2												
2/2	2.47	10.42	164.9	191.9	46.7	52.2	24.4	26.2	0.62	0.48	7.26	6.01
2/2	3.26	13.20	113.9	123.7	29.5	31.4	17.3	18.3	0.47	0.42	3.05	3.44
3/2	3.42	11.41	145.4	138.4	64.8	55.7	32.1	30.1	0.44	0.41	6.48	5.85
3/2	2.02	13.58	198.0	180.3	89.0	75.2	49.5	40.0	0.47	0.50	10.94	8.96
3/2	2.34	9.69	209.6	211.8	99.1	106.7	54.1	58.9	0.34	0.31	8.17	8.41
3/2	3.31	10.81	158.3	166.9	86.9	94.3	48.0	49.1	0.40	0.38	9.34	8.74
3/2	2.25	11.83	147.6	179.0	70.7	88.7	35.6	49.3	0.42	0.35	6.91	8.01
4/2	2.81	13.71	196.3	219.3	87.1	109.4	45.8	56.0	0.46	0.37	9.86	9.53
Mean	2.73	11.83	166.8	176.4	71.7	76.7	38.3	41.0	0.45	0.40	7.75	7.37
± SE	± 0.19	± 0.54[b]	± 11.5	± 11.7	± 8.4	± 9.9	± 4.6	± 5.2	± 0.03	± 0.022[b]	± 0.86	± 0.73
E3												
3/3	1.43	13.18	178.5	200.2	92.8	114.6	47.7	55.5	0.37	0.34	7.99	8.54
3/3	1.39	12.42	262.5	270.8	168.5	171.8	86.1	88.7	0.23	0.19	9.22	7.82
3/3	3.63	11.33	230.8	236.1	141.1	148.1	71.5	76.3	0.32	0.29	11.21	10.41
3/3	2.68	9.20	183.6	197.0	100.0	112.6	53.2	53.7	0.33	0.29	7.74	7.01
3/3	2.27	8.64	260.9	281.5	172.9	182.0	88.2	98.3	0.30	0.29	12.07	13.30
3/3	2.47	6.77	201.2	220.3	111.3	126.0	61.7	80.1	0.37	0.31	10.26	11.47
3/3	4.72	15.76	186.1	225.8	105.3	131.2	51.0	67.5	0.32	0.31	7.69	9.67
3/3	4.18	17.15	189.7	219.8	91.8	115.3	44.5	56.0	0.29	0.31	6.31	8.50
3/3	1.12	5.29	218.7	239.2	105.3	108.8	68.1	64.8	0.28	0.25	8.38	7.50
Mean	2.65	11.08	212.4	232.3	121.0	134.5	63.5	71.2	0.31	0.29	8.98	9.36
± SE	± 0.43	± 1.33[b]	± 10.9[a]	± 9.6[a,b]	± 10.5[a]	± 9.0[a,b]	± 5.4[a]	± 5.2[a,b]	± 0.01[a]	± 0.01[a,b]	± 0.62	± 0.69
E4												
4/3	2.57	8.68	256.6	260.6	153.8	155.5	76.4	80.3	0.27	0.25	9.38	9.40
4/3	2.30	4.85	218.6	226.5	130.6	138.0	68.5	75.9	0.41	0.30	13.25	10.71
4/3	2.85	17.81	184.3	215.9	95.5	124.1	49.6	61.0	0.37	0.31	8.59	8.71
4/3	4.18	13.42	184.0	199.9	102.3	115.7	54.1	60.8	0.33	0.33	8.66	9.30
4/3	1.13	7.39	187.5	199.5	109.9	123.8	57.9	62.4	0.25	0.29	6.90	8.28
4/3	1.39	8.65	175.2	206.8	100.3	133.9	59.8	66.8	0.33	0.29	9.41	8.85
4/3	2.11	12.40	175.4	207.8	90.0	107.1	49.4	52.3	0.32	0.27	7.02	6.42
4/3	2.23	10.14	232.3	257.5	118.0	141.7	59.6	68.7	0.29	0.23	7.74	7.36
4/3	1.94	11.05	185.9	220.1	106.1	131.0	62.3	67.3	0.29	0.28	8.60	9.18
4/3	3.66	11.56	213.8	231.2	120.0	122.6	60.1	61.0	0.31	0.32	8.85	9.09
4/3	1.79	11.28	142.7	169.8	71.4	73.4	37.1	36.5	0.35	0.30	5.84	5.06
4/4	4.29	14.37	240.9	332.3	144.5	214.1	81.5	101.4	0.26	0.19	9.90	8.93
Mean	2.54	10.97	199.8	227.3	111.8	131.7	59.7	66.2	0.31	0.28	8.68	8.44
± SE	± 0.30	± 0.99[b]	± 9.5[a]	± 11.9[a,b]	± 6.7[a]	± 9.5[a,b]	± 3.5[a]	± 4.5[a,b]	± 0.01[a]	± 0.01[a,b]	± 0.54	± 0.43
All (n = 29)												
Mean	2.63	11.24	194.6	214.8	103.6	117.4	55.0	60.8	0.35	0.32	8.52	8.43
± SE	± 0.18	± 0.58[b]	± 6.8	± 7.8[b]	± 6.0	± 7.2[b]	± 3.1	± 3.6[b]	± 0.016	± 0.01[b]	± 0.38	± 0.36

FCR, fractional catabolic rate; PR, production rate.
[a]Significantly different from apoE2.
[b]Significantly different from low cholesterol intake.

the subjects with ϵ4 allele but the changes were not significantly different from those in the subjects with ϵ2 allele (+2.9 ± 0.2 and −1.6 ± 1.1 mg/kg per day, respectively).

DISCUSSION

Serum levels of lipids

The present investigation is the first study in which serum lipid levels, cholesterol absorption, sterol balance, and LDL apoB kinetics have been studied simultaneously in humans during dietary interventions. The results revealed that by increasing daily cholesterol intake from a low level of 210 mg/day to 880 mg/day, elevations were obtained in serum levels of TC, LDL-C, and LDL apoB of 10, 13, and 9%, respectively, in a home-living population. The overall increase in total cholesterol was 21 mg/dl, a change that was clearly larger than the expected 14 mg/dl on the basis of the formula of Keys, Andersson, and Grande (43). This discrepancy may have been due to the

TABLE 4. Changes in different variables of cholesterol and LDL apoB metabolism by high cholesterol diet

Variables	All n = 29	ApoE2 n = 8	ApoE3 n = 9	ApoE4 n = 12
LDL cholesterol, mg/dl	+13.82 ± 2.87[b]	+4.98 ± 4.32	+13.50 ± 2.86[a,b]	+19.96 ± 5.48[a,b]
LDL apoB, mg/dl[c]	+5.80 ± 1.22[b]	+2.62 ± 2.53	+7.65 ± 2.41[a,b]	+6.53 ± 1.56[a,b]
FCR, pools/day	−0.036 ± 0.007[b]	−0.051 ± 0.018[b]	−0.025 ± 0.008[b]	−0.035 ± 0.011[b]
PR, mg/kg/day	−0.09 ± 0.21	−0.38 ± 0.34	0.37 ± 0.45	−0.24 ± 0.29
Cholesterol absorption, %	−3.08 ± 1.27[b]	−2.85 ± 1.96	−4.98 ± 2.51	−1.82 ± 2.10
Fecal bile acids, mg/kg/day	+0.88 ± 0.29[b]	+1.40 ± 0.56[b]	+0.28 ± 0.50	+0.99 ± 0.47
Cholesterol synthesis, mg/kg/day	−0.78 ± 0.72	−1.63 ± 1.08	−1.52 ± 1.29	−0.23 ± 1.30

Values given as mean ± SE.
[a]Significantly different from apoE2.
[b]Significantly different from low cholesterol intake.
[c]Analyzed with isopropyl alcohol precipitation (refs. 29, 30).

TABLE 5. Cholesterol absorption, synthesis and fecal steroids during low and high cholesterol intake

Subjects, ApoE Phenotype	Cholesterol Absorption		Fecal Steroids: Bile Acids		Fecal Steroids: Neutral Sterols		Cholesterol Synthesis	
	Low	High	Low	High	Low	High	Low	High
	%		mg/kg/day				mg/kg/day	
E2								
2/2	40.7	31.3	4.9	9.0	8.3	11.6	10.8	10.2
2/2	33.9	31.5	4.5	6.2	8.7	15.9	10.0	8.9
3/2	28.9	37.3	6.7	7.6	10.1	20.8	13.4	17.0
3/2	33.1	24.2	5.4	7.4	4.3	9.8	7.7	3.6
3/2	34.5	34.5	9.2	7.5	11.8	14.8	18.6	12.6
3/2	40.0	35.9	5.7	6.7	10.3	13.7	12.7	9.5
3/2	39.7	36.4	4.2	5.5	6.6	16.1	8.5	9.7
4/2	37.5	34.4	11.3	13.4	9.7	15.7	18.2	15.4
Mean ± SE	36.0 ± 1.5	33.2 ± 1.5	6.5 ± 0.9	7.9 ± 0.9[b]	8.7 ± 0.8	14.8 ± 1.2[b]	12.5 ± 1.4	10.9 ± 1.5
E3								
3/3	45.1	28.2	7.5	11.0	7.0	17.9	13.1	15.7
3/3	44.1	41.4	5.0	4.6	6.6	16.7	10.2	8.8
3/3	33.6	37.5	5.3	5.9	10.5	19.9	12.1	14.4
3/3	48.5	30.8	4.1	3.2	5.9	10.1	7.4	4.2
3/3	45.9	43.5	4.7	3.3	7.8	11.9	10.2	6.5
3/3	38.8	38.4	6.7	6.5	7.6	8.5	11.9	8.3
3/3	38.7	39.9	7.5	6.8	10.5	13.6	13.0	4.6
3/3	36.8	31.7	5.7	6.9	8.8	18.2	10.3	8.0
3/3	39.0	34.3	3.1	3.9	7.3	14.5	9.2	13.0
Mean ± SE	41.2 ± 1.6[a]	36.2 ± 1.7	5.5 ± 0.5	5.8 ± 0.8	8.0 ± 0.5	14.6 ± 1.3[b]	10.8 ± 0.6	9.3 ± 1.4
E4								
4/3	34.7	41.9	2.5	3.7	6.7	12.0	6.6	7.1
4/3	40.7	35.7	5.2	5.1	9.0	10.9	11.9	11.1
4/3	53.1	48.9	7.5	8.8	5.9	18.8	10.5	9.7
4/3	44.8	27.7	9.7	9.6	6.4	12.9	11.9	9.1
4/3	23.7	28.7	4.8	6.2	9.5	14.7	13.2	13.5
4/3	36.2	32.5	3.8	8.0	10.7	17.8	13.1	17.2
4/3	45.6	38.4	2.9	6.7	7.9	25.0	8.7	19.3
4/3	39.6	37.2	5.4	4.7	4.8	11.8	7.9	6.3
4/3	29.7	25.6	3.2	4.0	8.0	15.0	9.2	7.9
4/3	35.0	44.5	4.3	5.5	9.6	16.0	10.2	10.0
4/3	34.0	38.2	9.6	9.5	7.2	15.0	15.0	13.3
4/4	53.0	49.0	4.3	3.2	5.8	8.0	5.8	−3.1
Mean ± SE	39.2 ± 2.6	37.3 ± 2.3	5.3 ± 0.7	6.2 ± 0.6	7.6 ± 0.5	14.8 ± 1.3[b]	10.3 ± 0.8	10.1 ± 1.6
All (n = 29)								
Mean ± SE	38.9 ± 1.3	35.8 ± 1.2[b]	5.7 ± 0.4	6.6 ± 0.4[b]	8.0 ± 0.3	14.7 ± 0.7[b]	11.1 ± 0.6	10.3 ± 0.9

[a]Significantly different from apoE2.
[b]Significantly different from low cholesterol intake.

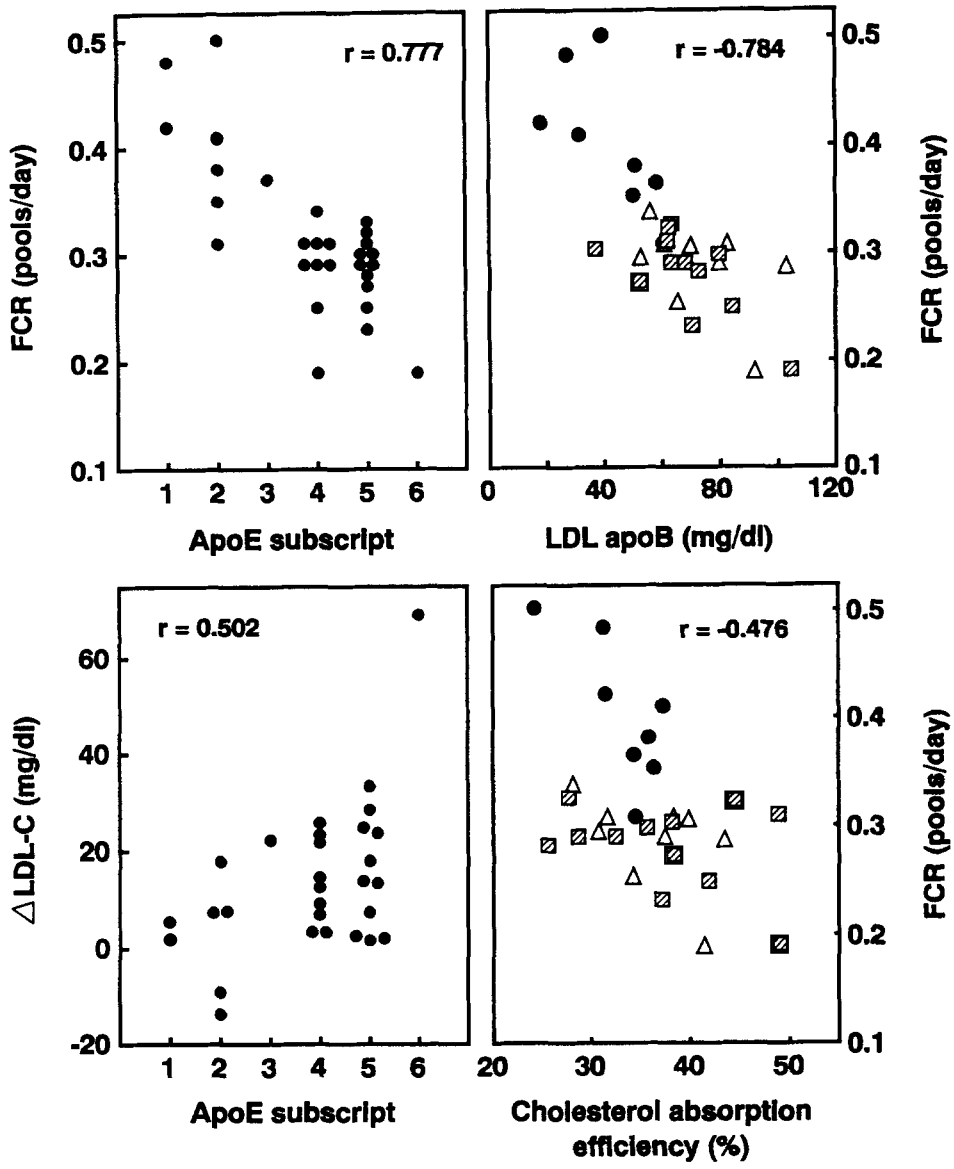

Fig. 1. Correlations between apoE subscript, change in LDL cholesterol, cholesterol absorption efficiency, and fractional catabolic rate of LDL apoB during high cholesterol intake. Left panels: 1, E2/2; 2, E3/2; 3, E4/2; 4, E3/3; 5, E4/3; and 6, E4/4. Right panels: closed circles, apoE2; shaded squares, apoE3; and open triangles, apoE4.

low basal cholesterol intake but may also suggest a vigorous response of serum cholesterol to dietary cholesterol among the Finns and could contribute to the nationwide elevation in serum cholesterol levels (44).

Responsiveness of serum cholesterol to high cholesterol intake and the mechanisms involved in this regulation have been widely studied. A low or normal baseline cholesterol level is a factor for nonresponsiveness (8). The present population exhibited similar relationship, even though the change in LDL-C by the high cholesterol intake was related to the LDL-C level only during the high, not low, cholesterol intake. In repeated cholesterol feeding studies a consistency of the response could be observed with a within-person standard deviation of 10.3 mg/dl of serum total cholesterol (45). The response of serum cholesterol can be related to different aspects of cholesterol metabolism, viz. absorption, synthesis, and conversion to bile acids, and to subsequent changes in LDL apoB metabolism, all together being finally contributed by genetic factors, e.g., by apoE phenotypes.

Cholesterol absorption

Despite the slightly decreased cholesterol absorption efficiency during the high cholesterol intake, the absolute amount of absorbed dietary cholesterol was increased four times, to an amount large enough to increase the serum cholesterol level. The increase in LDL cholesterol was, in fact, related to the absorbed dietary cholesterol. An in-

teresting example of apparent decrease in cholesterol absorption efficiency during cholesterol feeding is a patient who consumed 25 eggs a day but absorbed only 18% of cholesterol with virtually normal serum cholesterol (15). Dietary fat, which in a previous study (28) was positively associated with cholesterol absorption efficiency, was positively correlated with the absolute amount of cholesterol absorbed in the present study, a finding consistent with an earlier observation (46). Even though the amount of fat was intended to be constant during both dietary periods, the increment of egg yolk cholesterol resulted in a slight increase in fat intake with unaltered P/S ratios. However, the increase of dietary fat intake was not related to the increase in the LDL-C level.

Cholesterol absorption efficiency is partly genetically regulated with a wide variation from 24 to 74% (14). It differs only little between different populations, although extremely low values in some ethnic groups have been observed (13) perhaps due to a low fat intake. The basic mechanism by which increased dietary cholesterol intake reduces fractional cholesterol absorption in some (7, 11, 15, 16), including the present results but not in all studies (1, 13, 14, 47), could be due to increased dilution of labeled sterols used for absorption measurement by expanded intestinal pool size of cholesterol, resulting in reduced absorption of the label. In addition, micellar solubilization could be reduced and cholesterol uptake by the brush border membrane could be oversaturated provided cholesterol absorption is an active process (48). Cholesterol synthesis will be altered by impaired absorption, e.g., by neomycin (49, 50), suggesting that cholesterol absorption plays an important role in cholesterol homeostasis.

Bile acid synthesis

Dietary cholesterol usually has little effect on bile acid synthesis in humans. However, under normal conditions bile acid synthesis is negatively related to cholesterol absorption (14) and in some studies cholesterol feeding enhances bile acid synthesis (1, 15, 51). Those findings and the significant increase in bile acid synthesis during the high cholesterol intake in the present study suggest that enhanced bile acid synthesis also contributes to nonresponsiveness to high cholesterol intake—especially because the increase of bile acid output tended to be highest in the nonresponders.

Effective bile acid synthesis appears to prevent response to dietary cholesterol in animal studies also. For instance, hypercholesterolemia-resistant rabbits have a markedly higher fecal elimination of cholesterol as bile acids than rabbits developing hypercholesterolemia in response to dietary cholesterol (52).

Cholesterol synthesis

Another factor contributing to nonresponsiveness of serum cholesterol to dietary cholesterol is a down-regulation of cholesterol synthesis. In most cholesterol feeding studies cholesterol synthesis is actually reduced (1, 7, 11–13, 20). In the extensive study of McNamara et al. (7), the nonresponders were able to reduce cholesterol synthesis by 26%, while the reduction was only 12% in responders. On the other hand, Kern (15) described a patient who consumed 25 eggs daily without any raise in his serum TC, but cholesterol absorption was low, bile acid synthesis was twice the normal value, and cholesterol synthesis was of the same magnitude as in a control group. In the present free-living male population, a relatively long-term cholesterol feeding had no constant effect on cholesterol synthesis so that the increase in absolute cholesterol absorption was associated with enhanced fecal output of endogenous steroids, indicating increased cholesterol turnover rate mainly as enhanced bile acid synthesis. An increase in resecretion of dietary cholesterol can also occur (51, 53). Unaltered cholesterol synthesis appears to occur also in women during cholesterol feeding (16).

LDL apoB metabolism

The markedly increased cholesterol absorption during the high cholesterol diet was apparently large enough to load hepatic cells with cholesterol so that the receptor activity was down-regulated, FCR for apoB was reduced, and LDL-C increased. The inverse correlation between the absorption efficiency of cholesterol with the FCR for LDL apoB actually shows for the first time that intestinal cholesterol absorption and apoB/E receptor activity are closely related with each other in humans. Accordingly, the dietary cholesterol-induced increase in LDL-C was related to absorbed dietary cholesterol and FCR for LDL apoB. Dietary cholesterol has been known to reduce LDL apoB catabolism and increase its synthesis (4), and to down-regulate LDL apoB receptor activity in monocytes (2, 5) but not in fibroblasts (3).

ApoE phenotype

An important observation, evident also during the baseline period on liberal home diet (28, 54) and during the low cholesterol intake as well (28) and in E2 and E3 subjects (55), was a negative correlation of FCR for LDL apoB with apoE subscript and LDL-C or LDL apoB. Accordingly, subjects with apoE2 phenotype had the fastest catabolism of LDL apo-B and the lowest serum levels of LDL-C associated with low cholesterol absorption efficiency. In earlier studies it was shown that serum cholesterol of lowest deciles was associated with accelerated FCR for LDL apoB (56, 57), resembling subjects with apoE2 in the present study. The up-regulation of the hepatic apoB/E receptors in apoE2 subjects is assumed to compensate for the reduced cholesterol transport to the liver, which is due to low cholesterol absorption (22) and

insufficient chylomicron and VLDL remnant binding capacity to apoB/E receptors (58–61).

To study the contribution of genetic factors, the different apoE phenotype groups, including E2/2 and E4/4 subjects, were selected to be large enough to allow statistical treatment for different variables between various apoE phenotypes in the present series. Before and during cholesterol loading, the serum levels of TC, LDL-C, and LDL apoB were lower in the apoE2 than in the E3 and E4 subjects, and the cholesterol values and the apoE subscripts were significantly related with each other. The responders to increased dietary cholesterol were the subjects with $\epsilon 3$ and $\epsilon 4$ alleles but not those with $\epsilon 2$ allele, so that the increase of LDL-C was significantly related to the apoE subscript (Fig. 1). In contrast to these findings on a low, modestly unsaturated fat diet, the serum cholesterol response to dietary cholesterol has been poorly related to the apoE phenotypes during high fat diets (27, 62). The study by Boerwinkle et al. (62) did not include homozygous E2 and E4 subjects. Many of the individuals with $\epsilon 3$ and $\epsilon 4$ alleles of the present series had only a small increase in LDL-C (Fig. 1) and variables of cholesterol and LDL metabolism were poorly related to different LDL responses in different apoE phenotypes. The high bile acid synthesis and its significant increase were major factors involved in the nonresponsiveness of the individuals with $\epsilon 2$ allele to dietary cholesterol. Tendencies to reduce cholesterol synthesis, to increase less dietary cholesterol absorption, and even to decrease synthesis of LDL apoB might be additional contributory factors.

Our findings suggest that, analogous to animal experiments (52), genetic factors (apoE phenotypes at least) contribute to responsiveness to dietary cholesterol in humans. Low cholesterol absorption efficiency, enhanced bile acid synthesis, and effective elimination of endogenous cholesterol to feces appear to be factors upregulating LDL apoB receptors so that LDL-C is low under normal conditions and during cholesterol feeding, especially in subjects with apoE2 phenotypes. On the other hand, subjects with E4 allele, especially the homozygous ones, appear to be sensitive to dietary cholesterol and fat (28). Since the prevalence of the E4 allele is exceptionally high in the Finnish population (21), both dietary and genetic factors could contribute strongly to the high cholesterol concentration and the high incidence of coronary heart disease recorded in international comparisons (44).

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