# Efficiency of intestinal cholesterol absorption in humans is not related to apoE phenotype

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**Abstract** **The present study investigated the role of apolipoprotein E (apoE) phenotype on intestinal cholesterol absorption and cholesterol synthesis. Studies were carried out in eight subjects homozygous for the apoE4 and 12 subjects homozygous for the E2 allele (six normocholesterolemic volunteers and six patients with type III hyperlipoproteinemia). Cholesterol absorption did not differ between the three groups of subjects and averaged 38 ± 2% (mean ± SEM) in normolipemic E2/2, 37 ± 4% in type III hyperlipemic E2/2, and 41 ± 3% in E4/4 subjects, respectively. Dietary intake of fat and cholesterol had no influence on cholesterol absorption efficiency. A positive correlation between efficiency of cholesterol absorption and the ratio of campesterol to cholesterol in plasma, an indirect marker for cholesterol absorption, was observed after combining the results of the three groups ($r = 0.504$; $P < 0.02$). Bile acid and total cholesterol synthesis were also not affected by the different apoE alleles, but the well-known relationship between body weight and cholesterol synthesis was noticed ($r = 0.574$; $P < 0.01$). Thus, the present study provides evidence that the efficiency of intestinal absorption and synthesis of cholesterol in humans are not related to the apoE phenotype.**—von Bergmann, K., D. Lütjohann, B. Lindenthal, and A. Steinmetz. **Efficiency of intestinal cholesterol absorption in humans is not related to apoE phenotype.** ***J. Lipid Res.*** **2003.** 44: **193–197.**

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The polymorphism of the human apolipoprotein E (apoE) is determined by three common alleles, ε2, ε3, and ε4, resulting in six phenotypes (1). ApoE alleles strongly affect total cholesterol, LDL-cholesterol, apoB, and apoE concentrations in the population (2–6). The influence of different isoforms of apoE on plasma cholesterol concentrations has been explained by several mechanisms: *A*) receptor-binding affinities of the different apoE containing lipoproteins (7), *B*) dietary fat clearance (8), *C*) difference in the clearance of LDL apoB (9), and *D*) efficiency of intestinal cholesterol absorption (10–12). A higher intestinal cholesterol absorption efficiency was shown in subjects with at least one ε4 allele as compared with subjects homozygous for ε3 allele. The apoE3/3 subjects had higher cholesterol absorption than those homo- or heterozygous for the E2 allele (10). However, only four patients with the apoE2 isoform (one homozygous for the ε2 allele), and only one subject homozygous for the ε4 allele were studied. In another study, Miettinen et al. (12) found a significant higher absorption in subjects with apoε3 and apoε4 compared with those with apoε2 alleles. This difference disappeared on a low fat low cholesterol diet. Again, only two subjects were homozygous for the apoε2 and ε4 allele, respectively. Thus, the role of apoE phenotype on cholesterol absorption efficiency has not been studied systematically. We therefore measured cholesterol absorption efficiency together with cholesterol and bile acid synthesis in subjects homozygous for the ε4 and ε2 alleles. The latter group was divided into subjects with normal plasma cholesterol and triglycerides, and those patients with familial type III hyperlipoproteinemia. Concomitantly, we measured the plant sterol campesterol in plasma of all subjects. Intestinal absorption of dietary campesterol is directly related to cholesterol absorption (13) and the ratio of plasma campesterol to cholesterol is an indirect marker of the rate of cholesterol absorption (14).

## MATERIALS AND METHODS

### Experimental design and subjects

This study was carried out with volunteers and patients attending the outpatient clinics of the Department of Internal Medicine, University of Marburg, and the Department of Clinical Pharmacology, University of Bonn, Germany. The primary aim was to compare the cholesterol absorption of subjects with

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TABLE 1. Clinical characteristics and plasma lipid concentrations of normolipemic and hyperlipemic E2/E2 and E4/E4 carriers

| | Apolipoprotein E2/2 (normolipemic, n = 6) | Apolipoprotein E2/2 (hyperlipemic, n = 6) | Apolipoprotein E4/4 (n = 8) |
|---|---|---|---|
| Age (years) | 32.3 ± 3.5 | 39.8 ± 4.4 | 46[e] ± 4.8 |
| Weight (kg) | 77.7 ± 6.0 | 91.2 ± 4.3 | 78.4 ± 4.4 |
| BMI[a] ($kg/m^2$) | 24.7 ± 1.2 | 29.4[b,d] ± 1.6 | 24.6 ± 1.1 |
| Triglycerides (mg/dl) | 140 ± 41 | 775[b,d] ± 253 | 274 ± 52 |
| Cholesterol (enzymatic) (mg/dl) | 155 ± 15 | 463[c,d] ± 70 | 273[f] ± 30 |
| Cholesterol (glc) (mg/dl) | 149 ± 11 | 460[c,d] ± 78 | 273[f] ± 27 |

Values are mean ± SEM.
[a] BMI, body mass index, [weight (kg)/height$^2$ (m)].
[b] Significantly different from normolipemic E2/2 carriers ($P < 0.05$).
[c] Significantly different from normolipemic E2/2 carriers ($P < 0.005$).
[d] Significantly different from E4/4 carriers ($P < 0.05$).
[e] Significantly different from normolipemic E2/2 carriers ($P < 0.05$).
[f] Significantly different from normolipemic E2/2 carriers ($P < 0.005$).

apoE2/2 with the absorption of apoE4/4 carriers. Six normolipemic volunteers (E2/2), six patients with type III hyperlipoproteinemia (E2/2), and eight subjects (E4/4) (six had hypercholesterolemia) participated in the study. None of the subjects received lipid-lowering drugs for at least 6 weeks prior to the study and none had a history of excessive alcohol intake, diabetes, or other endocrine disorders, renal disease, or diseases of the liver or gastrointestinal tract.

During the 1-week study, cholesterol absorption and fecal excretion of neutral and acidic sterols were measured. After an overnight fast, blood samples were collected for analysis of plasma lipids. Analysis of noncholesterol sterols was carried out at the beginning and the end of this week. The study was in accordance with the Helsinki Declaration for Human Studies and the protocol was approved by the local ethical committees. Written informed consent was obtained from all subjects before enrolment into the study.

### ApoE phenotyping

ApoE phenotypes were determined by isoelectric focusing of apolipoproteins with immunoblotting as previously described in detail (15).

### Plasma lipids

Blood samples were drawn after an overnight fast at the beginning and the end of the study week. Total plasma cholesterol and triglycerides were measured enzymatically using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany).

### Analysis of plasma cholesterol and noncholesterol sterols

Concentrations of cholesterol and noncholesterol sterols (cholestanol, lathosterol, and campesterol) in plasma were measured by a modified gas liquid chromatographic method as previously described (13). Briefly, to 0.1 ml of plasma, 50 μg of 5α-cholestane (Serva Feinbiochemica, Heidelberg, Germany) was added as internal standard. After alkaline hydrolysis, extraction with cyclohexane, and derivatisation to their trimethylsilyl-ethers, the sterols were separated on a 15 m dimethyl crosslinked column (Hewlett Packard HP, Böblingen, Germany).

### Cholesterol absorption

Cholesterol absorption was measured using the continuous isotope feeding method. For this purpose, subjects received stomach soluble capsules containing 3 mg of [26,26,26,27,27,27-$^2H_6$]cholesterol and 3 mg of [5,6,22,23-$^2H_4$]sitostanol (Medical Isotopes Inc.) tid for 7 days, and fecal samples were collected on days 5, 6, and 7. The samples were stored at −20°C until analysis. Fractional cholesterol absorption was then calculated from the fecal ratio of deuterium-labeled cholesterol and its bacterial degradation products coprostanol, coprostanone, and deuterium-labeled sitostanol, and from the ratio of deuterium-labeled cholesterol and sitostanol in the tracer capsules by gas liquid chromatography-mass spectrometry (GLC-MS), as previously described (16). The percentage of cholesterol absorption was calculated from the disappearance of cholesterol during the passage through the small intestine compared with sitostanol, which served as non-absorbable flow marker by the following formula:

TABLE 2. Cholesterol absorption in normolipemic and hyperlipemic E2/E2, and E4/E4 carriers

| | Cholesterol Absorption | | |
|---|---|---|---|
| Subject | Apolipoprotein E2/2 (Normolipemic, n = 6) | Apolipoprotein E2/2 (Hyperlipemic, n = 6) | Apolipoprotein E4/4 (n = 8) |
| | | %[a] | |
| 1 | 39.3 ± 2.0 | 32.3 ± 0.6 | 38.4 ± 2.5 |
| 2 | 40.3 ± 3.1 | 43.1 ± 1.3 | 48.9 ± 2.6 |
| 3 | 45.4 ± 2.1 | 29.8 ± 2.4 | 27.3 ± 4.2 |
| 4 | 31.2 ± 2.1 | 33.2 ± 3.8 | 33.2 ± 4.6 |
| 5 | 32.1 ± 3.2 | 52.5 ± 2.2 | 32.3 ± 4.0 |
| 6 | 38.5 ± 1.2 | 32.5 ± 5.3 | 49.8 ± 4.0 |
| 7 | | | 52.1 ± 3.3 |
| 8 | | | 43.3 ± 2.4 |
| Mean ± SEM | 37.8 ± 2.2 | 37.2 ± 3.6 | 40.8 ± 3.3 |

[a] Values are the mean ± SEM of 3 consecutive days.

TABLE 3. Dietary intake in normolipemic and hyperlipemic E2/E2, and E4/E4 carriers

| ApoE Type | Energy | Protein | Fat | Carbohydrates | Fiber | Protein | Fat | Carbohydrates |
|---|---|---|---|---|---|---|---|---|
| | *kcal/day* | | *g/day* | | | *% of total energy* | | |
| Normolipemic apoE2/2 (n = 6) | 2488 ± 658 | 82 ± 15 | 101 ± 30 | 259 ± 65 | 27 ± 6 | 14.2 ± 1.8 | 37.3 ± 8.3 | 43.7 ± 5.9 |
| Hyperlipemic apoE2/2 (n = 6) | 1968 ± 662 | 82 ± 18 | 76 ± 36 | 205 ± 58 | 41 ± 6 | 17.8 ± 2.8[a] | 34.2 ± 5.8 | 44.0 ± 6.2 |
| apoE4/4 (n = 8) | 2006 ± 440 | 83 ± 20 | 82 ± 18 | 195 ± 53 | 37 ± 7 | 17.1 ± 2.4[a] | 38.0 ± 6.7 | 40.1 ± 4.7 |

Values are mean ± SD (from one week dietary protocol).
[a] Significantly different from normolipemic E2/2 carriers ($P < 0.05$).

$$\text{Cholesterol absorption [\%]} = 100 \times \left(1 - \frac{([^2H_6]\text{-cholesterol}_{feces} + [^2H_6]\text{-coprostanol}_{feces})/[^2H_4]\text{-sitostanol}_{feces}}{[^2H_6]\text{-cholesterol}_{capsule}/[^2H_4]\text{-sitostanol}_{capsule}}\right)$$

The cholesterol absorption rates were derived from the mean of day 5, 6, and 7.

### Fecal excretion of neutral and acidic sterols, and cholesterol synthesis

Fecal excretion of neutral and acidic sterols was measured from the same fecal samples (days 5 to 7) collected for cholesterol absorption. For this purpose, each subject received sitostanol (30 mg tid) as a fecal recovery marker together with the stable isotope markers from day 1 to 7. Measurement of fecal neutral and acidic sterols together with sitostanol was performed by GLC (17). Daily fecal excretion rates of neutral and acidic sterols were then calculated as ratios to sitostanol in stools multiplied by the daily intake of sitostanol by the following formula:

$$\text{(1) Neutral sterol excretion (mg/d)} = \frac{(\text{cholesterol} + \text{coprostanol} + \text{coprostanone})\text{(mg/sample)}}{\text{sitostanol(mg/sample)}} \times \text{sitostanol intake (mg/d)}$$

$$\text{(2) Acidic sterol excretion (mg/d)} = \frac{\text{bile acids (mg/sample)}}{\text{sitostanol (mg/sample)}} \times \text{sitostanol intake (mg/d)}$$

The net cholesterol balance was calculated as the sum of daily excretion of fecal neutral plus acidic sterols minus the mean of dietary cholesterol intake during this week.

### Dietary intake of cholesterol

The subjects were trained by an experienced dietician to keep a food diary in which the kind, art of preparation, and amount of the food ingested was entered. All food and beverages were recorded. From the food diaries, the dietary cholesterol, protein, fat, carbohydrates, and fiber intakes were calculated with the computer program (18).

### Statistical analysis

Results are given as mean ± SEM. The difference in percentage cholesterol absorption, dietary cholesterol intake, fecal excretion neutral and acidic sterols, cholesterol synthesis, plasma lipoproteins and plasma noncholesterol sterols were analyzed using unpaired Student's *t*-test. Correlations were calculated by linear regression and stepwise regression analysis. $P < 0.05$ was considered significant. Data management and statistical analysis were performed using the statistical software SPSS/Windows (SPSS Inc.).

## RESULTS

The clinical characteristics and plasma concentrations of cholesterol and triglycerides of the subjects included in the study are presented in **Table 1**. Patients with type III hyperlipoproteinemia had a higher body weight compared with the two other groups, but only the body mass indexes were significantly different. By definition, plasma cholesterol and triglycerides in patients with type III hyperlipoproteinemia were higher compared with normolipemic subjects homozygous for ε2 alleles. They also had higher total cholesterol and triglycerides compared with homozygous ε4 carriers. The latter subjects had significantly higher plasma cholesterol concentrations than normolipemic volunteers homozygous for apoE2. Results of total cholesterol measured either enzymatically or by GLC were identical.

Individual results on cholesterol absorption in the three different groups of subjects are presented in **Table 2**. Cholesterol absorption ranged from 27% to 53%. In all apoE2/2 carriers the cholesterol absorption averaged 38% and was not significantly different from the absorption in subjects with apoE4/4 (41%). Furthermore, cholesterol absorption in normolipemic apoE2/2 volunteers was not different from the rates in patients with type III hyperlipopro-

TABLE 4. Cholesterol balance in normolipemic and hyperlipemic apolipoprotein E2/E2 and E4/E4 carriers

| Apolipoprotein | Dietary Cholesterol Intake | Fecal Excretion | | |
|---|---|---|---|---|
| | | Neutral Sterols | Acidic Sterols | Cholesterol Synthesis |
| | *mg/day* | | | |
| Normolipemic apoE2/2 (n = 6) | 294 ± 35 | 989 ± 148 | 681 ± 94 | 1376 ± 161 |
| Hyperlipemic apoE2/2 (n = 6) | 188[a] ± 29 | 902 ± 79 | 644 ± 75 | 1357 ± 136 |
| apoE4/4 (n = 8) | 179[a] ± 14 | 990 ± 79 | 658 ± 70 | 1469 ± 90 |

Values are the mean ± SEM.
[a] Significantly different from normolipemic E2/2 carriers.

teinemia. The normolipemic E2/2 subjects had a higher energy intake (~20%) compared with hyperlipedemic E2/2 and E4/4 carriers, the difference was not significant **(Table 3)**. They also had lower fiber (g/d) (ns) and protein intake in percentage of total calories ($P < 0.05$). However, the efficiency of intestinal cholesterol absorption was independent from the dietary habits, including dietary fat, fiber, and cholesterol intake, as well as age and body weight, although dietary intake of cholesterol was significantly higher in normolipemic E2/2 volunteers compared with the two other groups.

Bile acid and total cholesterol synthesis did not differ between the groups (**Table 4**). In all subjects, there was a positive correlation between BMI and cholesterol synthesis ($r = 0.626$; $P < 0.005$) and between body weight and cholesterol synthesis ($r = 0.574$; $P < 0.01$) (**Fig. 1**).

Plasma concentrations of cholestanol, lathosterol, campesterol, and their ratios to cholesterol are summarized in **Table 5**. Patients with type III hyperlipoproteinemia had significantly higher concentrations of all noncholesterol sterols compared with normolipemic apoE2/2 carriers. Plasma concentrations of lathosterol in patients with type III hyperlipoproteinemia were also higher compared with subjects with apoE4/4 alleles. In subjects homozygous for ε4, all noncholesterol plasma sterols were higher than in normolipemic E2/2 volunteers, but these differences were not statistically significant. However, the ratios of cholestanol, lathosterol, and campesterol to cholesterol did not differ between the three different subject groups. A significant correlation between the efficiency of cholesterol absorption and the ratio of campesterol to cholesterol in plasma was found in all subjects ($r = 0.504$; $P < 0.02$) (Fig. 1).

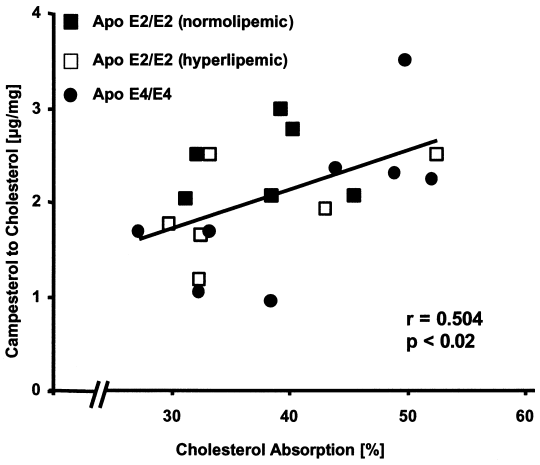


**Fig. 1.** The relationship between efficiency of cholesterol absorption and ratio of campesterol to cholesterol in plasma in 20 subjects (six normocholesterolemic apoE2/2, six type III hyperlipoproteinemia apoE2/2, and eight apoE4/4 subjects).

## DISCUSSION

Dietary cholesterol absorption in humans shows a wide variation (10, 16, 19–24). The reasons for this have not yet been elucidated in detail, although apoE isoforms have been implicated for some of these differences (10–12). However, no studies so far have systematically evaluated the role of apoE on cholesterol absorption efficiency. Therefore, the primary aim of the present study was to evaluate the role of the apoE on cholesterol absorption in humans. Participants of the present study were homozygous either for the ε2 or the ε4 allele. Furthermore, the apoE2 homozygous group consisted of six normocholesterolemic and six patients with familial type III hyperlipoproteinemia. We also compared bile acid and total cholesterol synthesis, as well as noncholesterol sterols in plasma. Thus, this is the first study where cholesterol absorption was compared in subjects homozygous for the apoε2 or the ε4 allele. Therefore, the present results are more likely to estimate the role of apoE on cholesterol absorption than previous studies.

The cholesterol absorption efficiency in the subjects of the present study ranged from 27% to 53%. This finding is in line with previous studies with healthy volunteers and patients with hypercholesterolemia using the identical continuous isotope feeding method with deuterium (16, 24) or radioactive isotopes labeled markers (11, 21). Repeated measurements of cholesterol absorption in the same individuals revealed constant absorption efficiency (16, 24), confirming not only the reproducibility of the method, but also the intraindividual consistency of dietary cholesterol absorption. The reason for interindividual differences have never been defined, although it has been postulated that apoE could account for some of them (10–12). In the present study, the absorption efficiency between subjects homozygous for apoE2 or E4 did not differ, neither did the ratio of campesterol to cholesterol. However, the ratio of campesterol to cholesterol in plasma was related to the efficiency of intestinal cholesterol absorption, thus confirming previous observations from Miettinen et al. (14).

It may be speculated that the absorption efficiency of higher amounts of dietary cholesterol that enter the intesti-

TABLE 5. Serum cholestanol, lathosterol, and campesterol and their ratios to cholesterol in normolipemic and hyperlipemic E2/E2, and E4/E4 carriers

| Apolipoprotein | Cholestanol | Lathosterol | Campesterol | Cholestanol/Cholesterol | Lathosterol/Cholesterol | Campesterol/Cholesterol |
|---|---|---|---|---|---|---|
| | *mg/dl* | | | | *µg/mg* | |
| Normolipemic apoE2/2 (n = 6) | 0.46 ± 0.20 | 0.34 ± 0.07 | 0.37 ± 0.04 | 2.74 ± 0.93 | 2.39 ± 0.58 | 2.41 ± 0.18 |
| Hyperlipemic apoE2/2 (N = 6) | 1.12[a] ± 0.27 | 1.00[a,b] ± 0.26 | 0.74[a] ± 0.11 | 2.72 ± 0.40 | 2.03 ± 0.40 | 1.92 ± 0.21 |
| apoE4/4 (n = 8) | 0.75 ± 0.21 | 0.44 ± 0.07 | 0.54 ± 0.09 | 2.88 ± 0.80 | 1.64 ± 0.24 | 1.97 ± 0.29 |

Values are mean ± SEM.
[a] Significantly different from normolipemic E2/2 ($P < 0.05$).
[b] Significantly different from E4/4 carriers ($P < 0.05$).

nal tract is regulated by apoE alleles. Indeed, in humans the absorption efficiency declines during higher cholesterol intake (25). Thus, the apparent difference between the present results and those obtained by Kesäniemi et al. (10) may be attributed to the amount of dietary cholesterol intake. The dietary intake of cholesterol in patients investigated by Kesäniemi et al. (10) was, on average, more than twice as high (~400 mg/day) as during the present study (~200 mg/day). On the other hand, Miettinen et al. (12) demonstrated that reducing the amount of dietary intake of fat by −37% and of cholesterol by −64%, the relationship between efficiency of cholesterol absorption and the apoE phenotype was lost. In contrast, Sehayek et al. (23) found no relationship between different apoE phenotypes and cholesterol absorption in a small number of subjects with a low (~200 mg/day) and high (~600 mg/day) cholesterol diet.

In the present study, no relationship between percentage of cholesterol absorption and dietary cholesterol was observed, although cholesterol intake ranged from 62 mg/day to 409 mg/day. Furthermore, we found no relationship between the dietary intake of different nutritions and cholesterol absorption. This lack of relationship might be due to the small numbers of subjects studied. Total cholesterol and bile acid synthesis during the present study also did not differ between individuals with the apoε2 and ε4 alleles. This is in line with the results of Kesäniemi et al. (10) and others (26, 27). The ratio of lathosterol to cholesterol, an indirect marker of hepatic and total cholesterol synthesis, was also not different between the different groups of subjects, confirming that cholesterol synthesis was not different between the groups.

In conclusion, the present study provides definitive evidence that the apoε focus is not involved in cholesterol absorption, at least during a dietary cholesterol intake in the range between 60 to 400 mg/day. This evidence is supplied directly by measurement of cholesterol absorption, and indirectly by the ratio of campesterol to cholesterol in plasma.

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## REFERENCES


1. Zannis, V. I., J. L. Breslow, G. Utermann, R. W. Mahley, R. I. Weisgraber, R. J. Havel, J. L. Goldstein, M. S. Brown, G. Schoenfeld, W. R. Hazzard, and C. B. Blum. 1982. Proposed nomenclatur of apoE isoproteins, apoE genotypes and phenotypes. *J. Lipid Res.* **23:** 911–914.
2. Utermann, G., N. Pruin, and A. Steinmetz. 1979. Polymorphism of apolipoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. *Clin. Genet.* **15:** 63–72.
3. Utermann, G., I. Kindermann, H. Kaffarnik, and A. Steinmetz. 1984. Apolipoprotein E phenotypes and hyperlipidemia. *Clin. Genet.* **65:** 232–236.
4. Assmann, G., G. Schmitz, H. J. Menzel, and H. Schulte. 1984. Apolipoprotein E polymorphism and hyperlipidemia. *Clin. Chem.* **30:** 641–643.
5. Ehnholm, C., M. Lukka, T. Kuusi, E. Nikkila, and G. Utermann. 1986. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27:** 227–235.
6. Davignon, J., R. E. Gregg, and C. F. Sing. 1988. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis.* **8:** 1–21.
7. Mahley, R. W., and Z. S. Ji. 1999. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* **40:** 1–16.
8. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J. Clin. Invest.* **80:** 1571–1577.
9. Gylling, H., K. Kontula, and T. A. Miettinen. 1995. Cholesterol absorption and metabolism and LDL kinetics in healthy men with different apoprotein E phenotypes and apoprotein B Xba and LDL receptor Pvu II genotypes. *Arterioscler. Thromb. Vasc. Biol.* **15:** 208–213.
10. Kesäniemi, Y. A., C. Ehnholm, and T. A. Miettinen. 1987. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J. Clin. Invest.* **80:** 578–581.
11. Miettinen, T. A., and Y. A. Kesäniemi. 1989. Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. *Am. J. Clin. Nutr.* **49:** 629–635.
12. Miettinen, T. A., H. Gylling, H. Vanhanen, and A. Ollus. 1992. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arterioscler. Thromb.* **12:** 1044–1052.
13. Heinemann, T., G. Axtmann, and K. von Bergmann. 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur. J. Clin. Invest.* **23:** 827–831.
14. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesäniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
15. Steinmetz, A. 1987. Phenotyping of human apolipoprotein E from whole blood plasma by immunoblotting. *J. Lipid Res.* **28:** 1364–1370.
16. Lütjohann D., C. O. Meese, J. R. Crouse, and K. von Bergmann. 1993. Evaluation of deuterated cholesterol and deuterated sitostanol for measurement of cholesterol absorption in humans. *J. Lipid Res.* **34:** 1039–1046.
17. Czubayko, F., B. Beumers, S. Lammsfuss, D. Lütjohann, and K. von Bergmann. 1991. A simplified micro-method for quantification of fecal excretion of neutral and acidic sterols for outpatient studies in humans. *J. Lipid Res.* **32:** 1861–1867.
18. Souci, S. W., W. Fachmann, and H. Kraut. 1990. Composition and nutrition tables. *In* Wissenschaftliche Verlagsgesellschaft mbH. Stuttgart, Germany.
19. Mok, H. Y., K. von Bergmann, and S. M. Grundy. 1979. Effects of continuous and intermittent feeding on biliary lipid outputs in man: application for measurements of intestinal absorption of cholesterol and bile acids. *J. Lipid Res.* **20:** 389–398.
20. von Bergmann, K., H. Y. Mok, W. G. Hardison, and S. M. Grundy. 1979. Cholesterol and bile acid metabolism in moderately advanced, stable cirrhosis of the liver. *Gastroenterology.* **77:** 1183–1192.
21. Crouse, J. R., and S. M. Grundy. 1978. Evaluation of a continuous isotope feeding method for measurement of cholesterol absorption in man. *J. Lipid Res.* **19:** 967–971.
22. Bosner, M. S., L. G. Lange, W. F. Stenson, and R. E. Ostlund, Jr. 1999. Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J. Lipid Res.* **40:** 302–308.
23. Sehayek, E., C. Nath, T. Heinemann, M. McGee, C. E. Seidman, P. Samuel, and J. L. Breslow. 1998. U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans. *J. Lipid Res.* **39:** 2415–2422.
24. Berthold, H. K., T. Sudhop, and K. von Bergmann. 1998. Effect of a garlic oil preparation on serum lipoproteins and cholesterol metabolism: a randomized controlled trial. *JAMA.* **279:** 1900–1902.
25. Ostlund, R. E., Jr., M. S. Bosner, and W. F. Stenson. 1999. Cholesterol absorption efficiency declines at moderate dietary doses in normal human subjects. *J. Lipid Res.* **40:** 1453–1458.
26. Jones, P. J., B. F. Main, and J. J. Frohlich. 1993. Response of cholesterol synthesis to cholesterol feeding in men with different apolipoprotein E genotypes. *Metabolism.* **42:** 1065–1071.
27. Palmer, R. H., A. V. Nichols, R. B. Dell, R. Ramakrishnan, F. T. Lindgren, E. L. Gong, C. B. Blum, and D. S. Goodman. 1986. Lack of relationship in humans of the parameters of body cholesterol metabolism with plasma levels of subfractions of HDL or LDL, or with apoE isoform phenotype. *J. Lipid Res.* **27:** 637–644.