

Associations of egg and cholesterol intakes with carotid intima-media thickness and risk of incident coronary artery disease according to apolipoprotein E phenotype in men: the Kuopio Ischaemic Heart Disease Risk Factor Study^{1,2}

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ABSTRACT

Background: In general populations, the effects of dietary cholesterol on blood cholesterol concentrations are modest. However, the relation is stronger in those with an $\epsilon 4$ allele in the apolipoprotein E gene (*APOE*). There is little information on the association between cholesterol intake and the risk of coronary artery disease (CAD) among those with the ApoE4 phenotype.

Objective: We investigated the associations of intakes of cholesterol and eggs, a major source of dietary cholesterol, with carotid intima-media thickness and the risk of incident CAD in middle-aged and older men from eastern Finland.

Design: The study included 1032 men aged 42–60 y in 1984–1989 at the baseline examinations of the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor Study. Data on common carotid artery intima-media thickness (CCA-IMT) were available for 846 men. Dietary intakes were assessed with 4-d food records. Associations with incident CAD and baseline CCA-IMT were analyzed by using Cox regression and ANCOVA, respectively.

Results: The ApoE4 phenotype was found in 32.5% of the men. During the average follow-up of 20.8 y, 230 CAD events occurred. Egg or cholesterol intakes were not associated with the risk of CAD. Each 1 additional egg (55 g)/d was associated with a multivariable-adjusted HR of 1.17 (95% CI: 0.85, 1.61) in the ApoE4 noncarriers and an HR of 0.93 (95% CI: 0.50, 1.72) in the ApoE4 carriers (*P*-interaction = 0.34). Each 100-mg/d higher cholesterol intake was associated with an HR of 1.04 (95% CI: 0.89, 1.22) in the ApoE4 noncarriers and an HR of 0.95 (95% CI: 0.73, 1.25) in the ApoE4 carriers (*P*-interaction = 0.81). Egg or cholesterol intakes were also not associated with increased CCA-IMT.

Conclusion: Egg or cholesterol intakes were not associated with increased CAD risk, even in ApoE4 carriers (i.e., in highly susceptible individuals). *Am J Clin Nutr* 2016;103:895–901.

Keywords apolipoproteins, atherosclerosis, cholesterol, coronary heart disease, diet

INTRODUCTION

The fundamental feeding experiments by Hegstedt et al. (1) and Keys et al. (2) led them to conclude that serum total

cholesterol is dependent on dietary cholesterol intake. This notion remained unchallenged for decades. However, over the past few years, several organizations and expert panels have concluded that there is insufficient evidence on the relation between dietary cholesterol intake and the risk of cardiovascular diseases (CVDs)⁶ in a general population, and in many dietary recommendations the suggestion to limit cholesterol intake has been discontinued (3–6).

In a general population, dietary cholesterol intake has only modest effects on serum total and LDL-cholesterol concentrations or the LDL- to HDL-cholesterol ratio and little effect on the risk of CVD (7). However, certain genetic factors can influence the effect of dietary cholesterol on serum lipids (8). One of these is the presence of the $\epsilon 4$ allele in the apolipoprotein E (*APOE*) gene, a key regulator in cholesterol and lipid metabolism (9). The *APOE* gene has 3 different alleles, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, which encode 3 common isoforms (E2, E3, and E4), so there are 6 different ApoE phenotypes: E2/2, E2/3, E2/4, E3/3, E3/4, and E4/4. Compared with those with the E3/3 phenotype, those with an $\epsilon 2$ allele have lower and those with an $\epsilon 4$ allele have higher total and LDL-cholesterol concentrations (9). The ApoE phenotype has been suggested to be one of the strongest genetic factors that affect serum lipid and lipoprotein variability (10). It has been estimated to account for as much as 16% of the genetic variation in serum LDL-cholesterol concentrations (10). One of the suggested

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² Supplemental Figure 1 and Supplemental Tables 1–5 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁶ Abbreviations used: ApoE, apolipoprotein E; CCA, common carotid artery; CCA-IMT, common carotid artery intima-media thickness; CRP, C-reactive protein; CVD, cardiovascular disease; IMT, intima-media thickness; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; TMAO, trimethylamine-*N*-oxide.

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mechanisms to explain this is the increased intestinal absorption of dietary cholesterol (11–14), although not all studies have observed such an effect (8). Therefore, the limitation of cholesterol intake may be more relevant in populations with higher ApoE4 frequency. The frequency of the ApoE4 phenotype varies markedly around the world, but Finland has one of the highest prevalences (15).

Eggs are a major contributor to cholesterol intake, with ~200 mg cholesterol in 1 medium-sized egg. Despite this, egg intake has not been associated with CVD risk in general populations (16, 17). However, little is known about whether an $\epsilon 4$ allele in ApoE modifies the association between egg or cholesterol intakes and the risk of CVD. We previously observed no association of egg intake with the risk of coronary artery disease (CAD) or carotid intima-media thickness (IMT), a predictor of CVD risk in this study population (18), but found an inverse association with the risk of type 2 diabetes in the eastern Finnish men from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) (19, 20). ApoE4 phenotype did not modify the associations of egg or cholesterol intakes with the risk of type 2 diabetes (20). In the current analysis we investigated whether ApoE4 phenotype could modify the associations of egg and cholesterol intakes with serum lipid profile, carotid IMT, and the risk of incident CAD.

METHODS

Study population

The KIHD was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a prospective, population-based, randomly selected sample of men from eastern Finland (21). The baseline examinations were carried out in 1984–1989. A total of 2682 men aged 42, 48, 54, or 60 y at baseline (82.9% of those eligible) were recruited in 2 cohorts (Supplemental Figure 1). The first cohort consisted of 1166 men who were 54 y old, enrolled in 1984–1986; and the second cohort included 1516 men who were 42, 48, 54, or 60 y old, enrolled in 1986–1989. The baseline examinations were followed by the 4-y examination round in 1991–1993, in which 1038 men from the second cohort (88% of those eligible) participated. The baseline characteristics of the entire study population have been described (22). The KIHD protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent for participation.

The ApoE phenotype was determined from blood samples of 1033 men who participated in the 4-y examinations and from 307 other men from the baseline examinations, for whom blood samples for phenotyping were available. Among these 1340 men, subjects with a history of CAD at baseline ($n = 302$) or with missing information on diet ($n = 6$) were excluded, leaving 1032 men for the analyses with incident CAD. Compared with those without data on ApoE phenotype, those with data on ApoE phenotype were, in general, healthier and had a more favorable lifestyle and dietary habits, although their serum lipid and lipoprotein profile was less favorable (Supplemental Table 1). Baseline common carotid artery IMT (CCA-IMT) measurements were available for 846 men. After exclusion of the outliers (values outside the mean \pm 1.5 times the IQR; $n = 34$), there were 812 men in the analyses of carotid atherosclerosis.

Assessment of dietary intakes

The consumption of foods at baseline was assessed with guided 4-d food records, of which one of the days was a weekend day, by using household measures. A picture book of common foods and dishes was used to help in estimation of portion sizes. The picture book contained 126 of the most common foods and drinks consumed in Finland, and for each food item the participant could choose from 3 to 5 commonly used portion sizes or describe the portion size in relation to those in the book. To further improve accuracy, instructions were given and completed food records were checked by a nutritionist together with the participant. Nutrient intakes were estimated by using the NUTRICA 2.5 software (Social Insurance Institution). The databank of the software is mainly based on Finnish values of the nutrient composition of foods. The egg consumption variable represents total egg consumption, including eggs in mixed dishes and recipes.

Assessment of carotid IMT

The extent and severity of carotid atherosclerosis were assessed by high-resolution B-mode ultrasonographic examination of the right and left common carotid arteries (CCAs) in a 1.0- to 1.5-cm section at the distal end of the CCA, proximal to the carotid bulb, as described earlier (18). Ultrasonographic examinations were conducted by one physician. All of the examinations were performed with the subject in a supine position. IMT, calculated as the mean distance between the intima-lumen and media-adventitia interfaces, was estimated at ~100 points in both the right and left CCAs. For the present study, 2 measures of IMT were used: 1) the mean IMT, calculated as the mean of all IMT estimates from the right and left CCAs and considered an overall measure of the atherosclerotic process, and 2) the maximal IMT, the average of the points of maximal thickness from the right and left CCAs and indicative of the depth of intrusion of IMT into the lumen in this part of the CCA. The variability of the CCA-IMT measurement in the KIHD has been shown to be relatively low. The interindividual CV was 10.5% for the first assessments by 4 observers, and the intrareader variability, described as the mean of the absolute difference between the first and third observations, was 8.3% of the mean IMT (18).

Other measurements

Venous blood samples were collected between 0800 and 1000 h at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h before providing the sample. Detailed descriptions of the determination of serum lipids and lipoproteins (23), assessment of medical history and medications at baseline (23), family history of diseases (23), smoking (23), alcohol intake (23), blood pressure (23), and physical activity (24) have been published. Serum C-reactive protein (CRP) was measured with an immunometric assay (Immuline High Sensitivity CRP Assay; Diagnostic Products Corporation). Education was assessed in years by using a self-administered questionnaire. The ApoE phenotype was determined from plasma with isoelectric focusing and immunoblotting techniques. Subjects who had the phenotype 3/4 or 4/4 were included in the ApoE4 group.

Ascertainment of follow-up events

Data on fatal and nonfatal CAD events from the beginning of the study to the end of the year 2012 were obtained by computer linkage to the national hospital discharge and death certificate registers. Diagnostic information was collected from hospitals and classified by using identical diagnostic criteria. Each suspected coronary event (International Classification of Diseases, 9th revision, codes 410–414 and International Classification of Diseases, 10th revision, codes I20–I25) was classified into 1) a definite acute myocardial infarction, 2) a probable acute myocardial infarction, 3) a typical acute chest pain episode of >20 min indicating CAD, 4) an ischemic cardiac arrest with successful resuscitation, or 5) no acute coronary event by a physician using the original patient records. Acute coronary events that did not lead to death during the following 24 h were considered as a nonfatal event. If a subject had multiple nonfatal CAD events during the follow-up or a nonfatal event followed by a fatal event, the first was considered the endpoint.

Statistical analysis

The univariate relations between egg and cholesterol intakes and baseline characteristics were assessed by means and linear regression (for continuous variables) or chi-square tests (for bivariate relations). Associations with carotid IMT and serum lipids and lipoproteins were analyzed with ANCOVA and linear regression. Cox proportional hazards regression models were used to estimate HRs of incident CAD. The validity of the proportional hazards assumption was evaluated by using Schoenfeld residuals. The confounders in the analyses were selected on the basis of established risk factors for CAD, previously published associations with CAD in the KIHHD, or on associations with exposures or outcomes in the present analysis. Model 1 included age (y), examination year, and energy intake (kcal/d). The multivariable model (model 2) included the variables in model 1 as well as BMI (kg/m²), diabetes (yes or no), hypertension (yes or no), family history of CAD (yes or no), smoking (never smoker, previous smoker, current smoker of <20 cigarettes/d, and current smoker of ≥20 cigarettes/d), education years, leisure-time physical activity (kcal/d), and intakes of alcohol (g/d), PUFAs (% of energy), SFAs (% of energy), dietary fiber (g/d), and fruit, berries, and vegetables (g/d). In the analyses of carotid IMT, the technical covariate, focusing depth, was also included. The cohort mean was used to replace missing values in covariates (<3%). Significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests with the use of a cross-product term. Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. All *P* values were 2-tailed ($\alpha = 0.05$). Data were analyzed by using SPSS 21.0 for Windows (IBM Corporation).

RESULTS

The average egg intake was 33 g/d (SD: 26 g/d; ~4 medium-sized eggs/wk), and the mean cholesterol intake was 398 mg/d (SD: 147 mg/d). Cholesterol intake from eggs (mean \pm SD: 110 \pm 85 mg/d) accounted for 27.7% of the total cholesterol intake. Fifteen percent (*n* = 155) consumed at least 1 medium-sized egg

(55 g)/d. Eight subjects did not consume eggs at all, and 3 subjects reported consuming egg whites only. Men with a higher egg intake were more physically active and less likely to smoke and to have diabetes (**Table 1**). They also had higher intakes of energy, fiber, and saturated fat and a lower polyunsaturated fat intake. Those with a higher cholesterol intake were younger, less physically active, and had a lower educational level. They also had higher intakes of energy, fiber, saturated fat, and mono-unsaturated fat and lower intakes of carbohydrates and poly-unsaturated fat.

Among the men, 28.6% had the ApoE 3/4 phenotype and 3.9% had the 4/4 phenotype (**Table 2**). During the average follow-up of 20.8 y (SD: 6.5 y), 230 men (22.3%) experienced a fatal or nonfatal CAD. Compared with the noncarriers, the ApoE4 carriers had a less favorable lipid profile (**Supplemental Table 2**). However, ApoE4 carriers did not have a higher CAD risk after adjustment for age and examination year (HR: 0.99; 95% CI: 0.75, 1.31), and multivariable adjustments did not change the association (HR: 0.99; 95% CI: 0.75, 1.31). Further adjustment for lipid-lowering medication use during the follow-up (46.0% in the ApoE4 carriers and 42.2% in the noncarriers) also did not appreciably affect the associations (HR: 1.02; 95% CI: 0.77, 1.36). There were no differences in the mean CCA-IMT (multivariable-adjusted difference \pm SEM: 0.003 \pm 0.08 mm; *P* = 0.67) or maximal CCA-IMT (difference \pm SEM: 0.004 \pm 0.011 mm; *P* = 0.70) between the ApoE4 carriers and noncarriers.

In a model that adjusted for multiple confounders, egg intake was associated with a generally more favorable lipid profile in the ApoE4 noncarriers, whereas no associations were found in the ApoE4 carriers (**Supplemental Table 3**). For example, egg intake was associated with a better total to HDL-cholesterol ratio and LDL- to HDL-cholesterol ratio in the ApoE4 noncarriers. Total cholesterol intake was not associated with serum lipids or lipoproteins, except for a trend toward a direct association with serum HDL cholesterol and a trend toward inverse associations with the total to HDL-cholesterol ratio and the LDL- to HDL-cholesterol ratio in the ApoE4 noncarriers (**Supplemental Table 4**).

There were no significant associations between either egg or cholesterol intake and CAD risk in the whole study population or in the analyses stratified by the ApoE4 phenotype (**Table 3**). Evaluated continuously, each 1 additional egg (55 g)/d was associated with a multivariable-adjusted HR of 1.17 (95% CI: 0.85, 1.61) in the ApoE4 noncarriers and an HR of 0.93 (95% CI: 0.50, 1.72) in the ApoE4 carriers (*P*-interaction = 0.34). Each 100-mg/d higher cholesterol intake was associated with an HR of 1.04 (95% CI: 0.89, 1.22) in the ApoE4 noncarriers and an HR of 0.95 (95% CI: 0.73, 1.25) in the ApoE4 carriers (*P*-interaction = 0.81). Adjustment for the use of lipid-lowering medication during the follow-up did not change the associations (data not shown). Egg or cholesterol intakes were also not associated with increased CCA-IMT (**Supplemental Table 5**).

Because the long follow-up time may attenuate the associations with the single-exposure assessment at baseline, in the sensitivity analyses we restricted the follow-up time to the first 10 y. However, we also did not find significant associations with the risk of CAD with this shorter follow-up time. For the ApoE4 noncarriers (46 CAD events), each 1-egg/d higher intake was associated with a multivariable-adjusted HR of 1.01 (95% CI: 0.54, 1.87) and each 100-mg/d higher cholesterol intake with an



TABLE 1

Baseline characteristics according to egg and cholesterol intakes in 1032 men from the KIH¹

| | Egg intake | | | Cholesterol intake | | |
|--|------------------------|--------------------------|------------------------|--------------------------|-----------------------------|--------------------------|
| | Tertile 1 (<19 g/d) | Tertile 2 (19–36 g/d) | Tertile 3 (>36 g/d) | Tertile 1 (<321 mg/d) | Tertile 2 (321–438 mg/d) | Tertile 3 (>438 mg/d) |
| Median intake ² | 11 | 26 | 52 | 267 | 373 | 522 |
| Age, y | 52.3 ± 6.4 | 51.6 ± 6.0 | 51.4 ± 6.0 | 52.6 ± 6.2 | 51.7 ± 6.4 | 51.0 ± 5.7* |
| Education, y | 9.2 ± 3.7 | 9.4 ± 3.5 | 9.3 ± 3.7 | 9.7 ± 4.1 | 9.3 ± 3.6 | 8.9 ± 3.2* |
| Marital status (married), % | 85 | 91 | 90 | 89 | 90 | 87 |
| Leisure-time physical activity, kcal/d | 125 ± 141 | 143 ± 152 | 150 ± 168* | 148 ± 143 | 142 ± 166 | 128 ± 152* |
| BMI, kg/m ² | 26.6 ± 3.3 | 26.6 ± 3.4 | 26.4 ± 2.9 | 26.6 ± 3.3 | 26.7 ± 3.3 | 26.3 ± 2.9 |
| Systolic blood pressure, mm Hg | 133 ± 15 | 133 ± 16 | 133 ± 16 | 134 ± 16 | 133 ± 15 | 132 ± 16 |
| Diastolic blood pressure, mm Hg | 89 ± 10 | 88 ± 10 | 88 ± 11 | 89 ± 10 | 88 ± 10 | 88 ± 11 |
| Current smoker, % | 36 | 28 | 24* | 25 | 32 | 31 |
| Diabetes, % | 6 | 3 | 2* | 4 | 4 | 3 |
| Hypertension, % | 59 | 52 | 53 | 56 | 59 | 49 |
| Family history of CAD, % | 52 | 46 | 48 | 51 | 47 | 47 |
| Lipid-lowering medication at baseline, % | 0.3 | 0.6 | 0.3 | 0.6 | 0.6 | 0 |
| Lipid-lowering medication during follow-up, % | 46 | 41 | 43 | 46 | 45 | 40 |
| Hypertension medication during follow-up, % | 68 | 68 | 67 | 69 | 67 | 67 |
| Blood glucose, mmol/L | 4.7 ± 1.0 | 4.6 ± 0.7 | 4.6 ± 0.8 | 4.6 ± 0.6 | 4.6 ± 0.8 | 4.7 ± 1.0 |
| Serum C-reactive protein, mg/L | 2.29 ± 3.28 | 2.19 ± 6.13 | 1.85 ± 2.63 | 2.43 ± 6.36 | 1.83 ± 2.40 | 2.07 ± 3.06 |
| Alcohol intake, g/wk | 79 ± 124 | 64 ± 103 | 73 ± 109 | 63 ± 90 | 79 ± 136 | 74 ± 105 |
| Dietary intakes | | | | | | |
| Energy, kcal/d | 2278 ± 549 | 2449 ± 580 | 2663 ± 591* | 2027 ± 407 | 2416 ± 397 | 2947 ± 557* |
| Protein, % of energy | 16.0 ± 2.8 | 15.9 ± 2.4 | 15.8 ± 2.6 | 16.1 ± 2.8 | 15.7 ± 2.3 | 15.8 ± 2.6 |
| Carbohydrates, % of energy | 42.8 ± 7.1 | 43.9 ± 5.9 | 42.8 ± 6.3 | 45.7 ± 6.4 | 42.9 ± 6.1 | 40.9 ± 5.8* |
| Fiber, g/d | 24.7 ± 9.1 | 26.3 ± 9.4 | 26.5 ± 8.8* | 23.8 ± 8.7 | 25.4 ± 8.3 | 28.4 ± 9.7* |
| SFAs, % of energy | 17.5 ± 4.4 | 17.4 ± 3.6 | 18.1 ± 3.8* | 15.4 ± 3.2 | 17.9 ± 3.6 | 19.7 ± 3.8* |
| PUFAs, % of energy | 4.8 ± 1.5 | 4.7 ± 1.4 | 4.5 ± 1.2* | 5.0 ± 1.5 | 4.6 ± 1.4 | 4.4 ± 1.2* |
| MUFAs, % of energy | 11.8 ± 2.3 | 11.6 ± 2.3 | 11.9 ± 2.1 | 11.4 ± 2.4 | 11.7 ± 2.2 | 12.1 ± 2.1* |
| trans fatty acids, % of energy | 1.1 ± 0.4 | 1.1 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.4 | 1.1 ± 0.4 | 1.0 ± 0.3 |
| Processed red meat, g/d | 72 ± 61 | 67 ± 64 | 73 ± 59 | 51 ± 47 | 71 ± 54 | 90 ± 74* |
| Fruit, berries, and vegetables, ³ g/d | 256 ± 168 | 262 ± 146 | 275 ± 154 | 276 ± 174 | 259 ± 144 | 258 ± 150 |

¹Values are means ± SDs unless otherwise indicated. **P*-trend ≤ 0.05 across tertiles. *P*-trend was assessed with linear regression (continuous variables) or chi-square test (bivariate relations). CAD, coronary artery disease; KIH¹, Kuopio Ischaemic Heart Disease Risk Factor Study.

²Median intakes are in grams per day for egg intake and milligrams per day for cholesterol intake.

³Excluding potatoes.

HR of 1.08 (95% CI: 0.82, 1.42). For the ApoE4 carriers (30 events), the respective HRs were 1.05 (95% CI: 0.40, 2.75) for each 1 additional egg/d and 0.96 (95% CI: 0.62, 1.47) for each 100-mg/d higher cholesterol intake. We also investigated the associations of egg and cholesterol intakes with CAD risk in those who did not start using lipid-lowering medications during the follow-up (*n* = 584). The consumption of each 1 egg/d was associated with a multivariable-adjusted HR of 1.13 (95% CI: 0.75, 1.71) in the ApoE4 noncarriers (100 events, 403 participants) and with an HR of 1.18 (95% CI: 0.52, 2.66) in the ApoE4 carriers (44 events, 181 participants). For each 100-mg higher cholesterol intake the HRs were 0.98 (95% CI: 0.79, 1.23) in the ApoE4 noncarriers and 0.97 (95% CI: 0.65, 1.45) in the ApoE4 carriers.

DISCUSSION

In this population-based cohort study in middle-aged and older men from eastern Finland, we found that higher egg or cholesterol intakes were not associated with the risk of incident CAD or with carotid IMT in the whole study population or in the ApoE ε4 carriers. There is limited evidence on the association between dietary cholesterol intake and the risk of CVD (7), and although

egg intake has been associated with a higher risk of CVD in patients with diabetes, no such association has been found in generally healthy populations (16, 17). Few epidemiologic studies have investigated the association between egg or cholesterol intakes and subclinical disease (19, 25–27), and only one study found increased carotid atherosclerosis with higher egg intake (25). However, in that study, important confounders, such as physical activity or other dietary factors besides eggs, were not accounted for (25), which limits the possibility to draw conclusions on the independent association of egg intake. In many study populations higher egg intakes tend to be associated with unhealthy lifestyle and dietary factors, such as smoking, lower physical activity, or a higher intake of processed red meat (28–30), which all are risk factors for CVD. In our study population, such associations were not observed (Table 1). In randomized controlled trials lasting from a few weeks to a few months, the addition of 2–3 eggs/d to a diet did not affect endothelial function (31–33). **Increasing egg intake also improved several CVD risk factors, such as increased formation of larger and less dense LDL and HDL particles (34, 35), decreased inflammatory markers (36–38), and improved glucose metabolism (35),** although not all trials found improvements in the risk

TABLE 2
Frequencies of the ApoE phenotypes among 1032 men from the KIH¹

| Phenotype | Frequency, <i>n</i> | Proportion, % |
|-----------|---------------------|---------------|
| 2/2 | 3 | 0.3 |
| 2/3 | 61 | 5.9 |
| 3/3 | 620 | 60.1 |
| 2/4 | 13 | 1.3 |
| 3/4 | 295 | 28.6 |
| 4/4 | 40 | 3.9 |

¹ApoE, apolipoprotein E; KIH¹, Kuopio Ischaemic Heart Disease Risk Factor Study.

factors (31–33, 39). Overall, egg or cholesterol intakes do not appear to be associated with adverse cardiovascular outcomes in general populations, and according to the results from our study, the associations are similar even in hyperresponders to dietary cholesterol (i.e., ApoE ε4 carriers).

In addition to dietary cholesterol, egg yolk is also a major source of choline (40), which is a source for trimethylamine-*N*-oxide (TMAO) production (41). TMAO was recently found to accelerate atherosclerosis in animal models and to be an independent risk factor for CVD in humans (41, 42), and increased egg intake has been shown to increase the production of TMAO

(42, 43). However, higher choline intake has also been inversely associated with inflammation (44) and, as noted above, egg intake has not been associated with CVD in general populations (16, 17). Eggs are an inexpensive and widely available source of several beneficial nutrients, such as high-quality protein, unsaturated fatty acids, vitamins, and minerals. Eggs are also a good source of other bioactive compounds, such as lutein, zeaxanthin, and phospholipids, which can have beneficial effects on inflammation, lipid oxidation, lipid metabolism, and atherosclerosis progression (45–48). Therefore, the health effects of eggs, or any other food, cannot be reliably determined by a single nutrient in the food, such as cholesterol or choline in eggs. This emphasizes the need to investigate the impact of whole foods, rather than individual nutrients or food components, on health.

The strength of our study is the detailed information on dietary intakes, which were assessed by using a 4-d food record and which included data on eggs in mixed dishes and recipes. The proportion of the ApoE ε4 carriers was also larger than in most other populations, which enabled us to investigate the associations between egg and cholesterol intakes and risk of CVD in this subpopulation with increased serum lipid response to dietary cholesterol. The other strengths were the population-based recruitment, extensive database of potential confounders, detailed classification of the CAD events, and virtually no loss to follow-up.

TABLE 3
Risk of coronary artery disease by tertile of egg and cholesterol intake among 1032 men from the KIH¹

| | Intake | | | <i>P</i> -trend | <i>P</i> -interaction |
|-----------------------------------|---------------|--------------------------------|-------------------|-----------------|-----------------------|
| | Tertile 1 | Tertile 2 | Tertile 3 | | |
| Egg intake (median), g/d | <19 (11) | 19–36 (26) | >36 (52) | | |
| All participants | | | | | |
| Number of events/participants (%) | 78/342 (22.8) | 69/346 (19.9) | 83/344 (24.1) | | |
| Model 1 ² | 1 | 0.84 (0.61, 1.17) ³ | 1.00 (0.72, 1.39) | 0.84 | |
| Model 2 ⁴ | 1 | 0.96 (0.69, 1.34) | 1.18 (0.85, 1.66) | 0.27 | |
| ApoE4 noncarriers | | | | | |
| Number of events/participants (%) | 49/224 (21.9) | 49/244 (20.1) | 58/229 (25.3) | | |
| Model 1 ² | 1 | 0.85 (0.57, 1.27) | 1.13 (0.75, 1.70) | 0.41 | |
| Model 2 ⁴ | 1 | 0.94 (0.63, 1.42) | 1.31 (0.86, 1.99) | 0.16 | |
| ApoE4 carriers | | | | | |
| Number of events/participants (%) | 29/118 (24.6) | 20/102 (19.6) | 25/115 (21.7) | | |
| Model 1 ² | 1 | 0.85 (0.48, 1.52) | 0.78 (0.44, 1.36) | 0.39 | 0.27 |
| Model 2 ⁴ | 1 | 1.05 (0.57, 1.91) | 1.10 (0.61, 1.99) | 0.74 | 0.35 |
| Cholesterol intake (median), mg/d | <321 (267) | 321–438 (373) | >438 (522) | | |
| All participants | | | | | |
| Number of events/participants (%) | 75/344 (21.2) | 82/344 (23.8) | 73/344 (21.2) | | |
| Model 1 ² | 1 | 1.11 (0.79, 1.56) | 1.00 (0.66, 1.54) | 0.96 | |
| Model 2 ⁴ | 1 | 1.05 (0.74, 1.50) | 1.00 (0.63, 1.58) | 0.96 | |
| ApoE4 noncarriers | | | | | |
| Number of events/participants (%) | 50/225 (22.2) | 56/239 (23.4) | 50/233 (21.5) | | |
| Model 1 ² | 1 | 1.04 (0.69, 1.56) | 0.94 (0.56, 1.57) | 0.78 | |
| Model 2 ⁴ | 1 | 0.99 (0.65, 1.52) | 0.93 (0.54, 1.61) | 0.78 | |
| ApoE4 carriers | | | | | |
| Number of events/participants (%) | 25/119 (21.0) | 26/105 (24.8) | 23/111 (20.7) | | |
| Model 1 ² | 1 | 1.31 (0.72, 2.38) | 1.17 (0.52, 2.62) | 0.67 | 0.92 |
| Model 2 ⁴ | 1 | 1.22 (0.64, 2.34) | 1.14 (0.46, 2.83) | 0.77 | 0.74 |

¹Cox proportional hazards regression models were used to obtain HRs and 95% CIs. ApoE, apolipoprotein E; KIH¹, Kuopio Ischaemic Heart Disease Risk Factor Study.

²Model 1 was adjusted for age, examination year, and energy intake.

³HR; 95% CI in parentheses (all such values).

⁴Model 2 was adjusted for variables as in model 1 and for smoking, BMI, diabetes, hypertension, leisure-time physical activity, coronary artery disease history in close relatives, education, and intakes of alcohol, fruit, berries, vegetables, fiber, PUFAs, and SFAs.

The potential limitation of the study was the rather small number of participants, which limited the power to find associations with incident CAD. Dietary habits were assessed only at baseline, which may have attenuated the associations with incident CAD during the long follow-up. Average egg consumption has remained relatively stable in Finland during the past 40 y (49), but cholesterol intake has decreased (50). However, egg or cholesterol intakes were not associated with increased carotid atherosclerosis at baseline, and the associations with incident CAD were not appreciably different with a shorter, 10-y follow-up, which supports the lack of association with incident CAD during the 21-y follow-up. The results are similar to those in another study, which found no significant association between egg intake and the risk of CAD in the analyses that assessed either the recent intake or cumulatively updated intake during the 8- to 14-y follow-up (28). Because of the limited study size, we were unable to separately investigate the impact of ApoE ϵ 4 homozygosity (ApoE 4/4), which may have the greatest impact on cholesterol absorption in ApoE phenotypes (13). The median egg intake in the highest tertile was 52 g/d, \sim 1 medium-sized egg, so our findings may not be generalizable to higher intakes. We did not have information on the preparation methods for eggs, so we were unable to investigate whether the associations would be similar for boiled and fried eggs. Our study population included only a few participants with type 2 diabetes, so we were unable to investigate whether the associations would be different in patients with diabetes, as has been suggested (16, 17). Although those with data on the ApoE phenotype had a less favorable lipid profile, they were, in general, healthier and had more favorable lifestyle factors than the rest of the KIHHD cohort. Therefore, the associations may not be generalizable to a more heterogeneous population. Although increased carotid IMT has been shown to predict CAD in the KIHHD, it may not indicate atherosclerosis progression, which is more characterized by focal lesions. Finally, because our study included only middle-aged and older men, the results may not be generalizable to other age groups or to women.

In conclusion, in the present study we examined the association between egg and cholesterol intakes and the risk of CAD and carotid IMT in a population with an exceptionally high prevalence of the ApoE ϵ 4 allele. We did not find any indications of the relation of moderate egg consumption (up to 1 egg/d) or moderate-to-high dietary cholesterol intake with increased CVD risk, even in these highly susceptible individuals. Hence, although our results are based on a rather small population, the findings suggest that removal of the recommendation to limit dietary cholesterol intake (including egg consumption) does not involve a marked risk for population health.

The authors' responsibilities were as follows—JKV, JM, HEKV, TTK, SV, and T-PT: acquired the data and designed and conducted the research; JKV: analyzed data, drafted the manuscript, and had primary responsibility for final content; JM, HEKV, MF, JTS, TTK, SV, and T-PT: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. MF received a research grant from Fazer Finland; JTS is the Chief Executive Officer of MAS-Metabolic Analytical Services Oy. The other authors reported no conflicts of interest.

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