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 ε4 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 ε4 allele in the apolipoprotein E (*APOE*) gene, a key regulator in cholesterol and lipid metabolism (9). The *APOE* gene has 3 different alleles, ε2, ε3 and ε4, 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 ε2 allele have lower and those with an ε4 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 ε4 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 ± 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 (Immulite 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 KIHD, 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/m^2), 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 (α = 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 ± SD: 110 ± 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 monounsaturated fat and lower intakes of carbohydrates and polyunsaturated 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 ± SEM: 0.003 ± 0.08 mm; P = 0.67) or maximal CCA-IMT (difference ± SEM: 0.004 ± 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 KIHD[1]

	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

[1]Values 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; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study.
[2]Median intakes are in grams per day for egg intake and milligrams per day for cholesterol intake.
[3]Excluding 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 KIHD[1]

Phenotype	Frequency, *n*	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

[1]ApoE, apolipoprotein E; KIHD, 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 KIHD[1]

	Intake				
	Tertile 1	Tertile 2	Tertile 3	*P*-trend	*P*-interaction
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

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

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

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

[4]Model 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 ε4 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, ∼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 KIHD 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 KIHD, 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 ε4 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.

REFERENCES

1. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965;17:281–95.
2. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. Metabolism 1965;14:759–65.
3. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, Lee IM, Lichtenstein AH, Loria CM, Millen BE, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014;129:S76–99.
4. Nordic Council of Ministers. Nordic nutrition recommendations 2012. Version current 25 March 2014 [cited 2015 Aug 30]. Available from: http://dx.doi.org/10.6027/Nord2014-002.
5. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Advisory Report to the Secretaries of the U.S. Department of Health and Human Services and the U.S. Department of Agriculture. Version current February 2015 [cited 2015 Aug 30]. Available from: http://www.health.gov/dietaryguidelines/2015-scientific-report/.
6. British Heart Foundation. Reducing your blood cholesterol. Version current 8 January 2014 [cited 2015 Aug 30]. Available from: https://www.bhf.org.uk/publications/heart-conditions/reducing-your-blood-cholesterol.
7. Berger S, Raman G, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr 2015;102:276–94.
8. Ordovas JM, Lopez-Miranda J, Mata P, Perez-Jimenez F, Lichtenstein AH, Schaefer EJ. Gene-diet interaction in determining plasma lipid response to dietary intervention. Atherosclerosis 1995;118:S11–27.
9. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1–21.
10. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 1985;37:268–85.
11. Kesäniemi YA, Ehnholm C, Miettinen TA. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. J Clin Invest 1987;80:578–81.
12. Gylling H, Miettinen TA. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. J Lipid Res 1992;33:1361–71.
13. Sarkkinen E, Korhonen M, Erkkila A, Ebeling T, Uusitupa M. Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and dietary cholesterol. Am J Clin Nutr 1998;68:1215–22.
14. Tammi A, Ronnemaa T, Rask-Nissila L, Miettinen TA, Gylling H, Valsta L, Viikari J, Valimaki I, Simell O. STRIP project (Special Turku Coronary Risk Factor Intervention Project for children). Apolipoprotein E phenotype regulates cholesterol absorption in healthy 13-month-old children—the STRIP Study. Pediatr Res 2001;50:688–91.
15. Gerdes LU, Klausen IC, Sihm I, Faergeman O. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genet Epidemiol 1992;9:155–67.
16. Rong Y, Chen L, Zhu T, Song Y, Yu M, Shan Z, Sands A, Hu FB, Liu L. Egg consumption and risk of coronary heart disease and stroke: dose-response meta-analysis of prospective cohort studies. BMJ 2013;346:e8539.
17. Shin JY, Xun P, Nakamura Y, He K. Egg consumption in relation to risk of cardiovascular disease and diabetes: a systematic review and meta-analysis. Am J Clin Nutr 2013;98:146–59.
18. Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993;87:II56–65.
19. Voutilainen S, Nurmi A, Mursu J, Tuomainen TP, Ruusunen A, Virtanen JK. Regular consumption of eggs does not affect carotid plaque area or risk of acute myocardial infarction in Finnish men. Atherosclerosis 2013;227:186–8.
20. Virtanen JK, Mursu J, Tuomainen TP, Virtanen HE, Voutilainen S. Egg consumption and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr 2015;101:1088–96.
21. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 1988;20:46–50.
22. Salonen JT, Salonen R, Seppanen K, Rauramaa R, Tuomilehto J. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation 1991;84:129–39.

23. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86: 803–11.
24. Lakka TA, Venäläinen JM, Rauramaa R, Salonen R, Tuomilehto J, Salonen JT. Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. N Engl J Med 1994;330:1549–54.
25. Spence JD, Jenkins DJ, Davignon J. Egg yolk consumption and carotid plaque. Atherosclerosis 2012;224:469–73.
26. Robbins JM, Petrone AB, Ellison RC, Hunt SC, Carr JJ, Heiss G, Arnett DK, Gaziano JM, Djousse L. Association of egg consumption and calcified atherosclerotic plaque in the coronary arteries: the NHLBI Family Heart Study. ESPEN J 2014;9:e131–5.
27. Goldberg S, Gardener H, Tiozzo E, Ying Kuen C, Elkind MS, Sacco RL, Rundek T. Egg consumption and carotid atherosclerosis in the Northern Manhattan Study. Atherosclerosis 2014;235:273–80.
28. Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascherio A, Colditz GA, Rosner BA, Spiegelman D, Speizer FE, Sacks FM, et al. A prospective study of egg consumption and risk of cardiovascular disease in men and women. JAMA 1999;281:1387–94.
29. Djoussé L, Gaziano JM. Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. Am J Clin Nutr 2008;87:964–9.
30. Scrafford CG, Tran NL, Barraj LM, Mink PJ. Egg consumption and CHD and stroke mortality: a prospective study of US adults. Public Health Nutr 2011;14:261–70.
31. Katz DL, Evans MA, Nawaz H, Njike VY, Chan W, Comerford BP, Hoxley ML. Egg consumption and endothelial function: a randomized controlled crossover trial. Int J Cardiol 2005;99:65–70.
32. Njike V, Faridi Z, Dutta S, Gonzalez-Simon AL, Katz DL. Daily egg consumption in hyperlipidemic adults—effects on endothelial function and cardiovascular risk. Nutr J 2010;9:28.
33. Katz DL, Gnanaraj J, Treu JA, Ma Y, Kavak Y, Njike VY. Effects of egg ingestion on endothelial function in adults with coronary artery disease: a randomized, controlled, crossover trial. Am Heart J 2015;169:162–9.
34. Mutungi G, Waters D, Ratliff J, Puglisi M, Clark RM, Volek JS, Fernandez ML. Eggs distinctly modulate plasma carotenoid and lipoprotein subclasses in adult men following a carbohydrate-restricted diet. J Nutr Biochem 2010;21:261–7.
35. Blesso CN, Andersen CJ, Barona J, Volek JS, Fernandez ML. Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome. Metabolism 2013;62:400–10.
36. Ratliff JC, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML. Eggs modulate the inflammatory response to carbohydrate restricted diets in overweight men. Nutr Metab (Lond) 2008;5:6.
37. Blesso CN, Andersen CJ, Barona J, Volk B, Volek JS, Fernandez ML. Effects of carbohydrate restriction and dietary cholesterol provided by eggs on clinical risk factors in metabolic syndrome. J Clin Lipidol 2013;7:463–71.
38. Andersen CJ, Lee JY, Blesso CN, Carr TP, Fernandez ML. Egg intake during carbohydrate restriction alters peripheral blood mononuclear cell inflammation and cholesterol homeostasis in metabolic syndrome. Nutrients 2014;6:2650–67.
39. Tannock LR, O'Brien KD, Knopp RH, Retzlaff B, Fish B, Wener MH, Kahn SE, Chait A. Cholesterol feeding increases C-reactive protein and serum amyloid A levels in lean insulin-sensitive subjects. Circulation 2005;111:3058–62.
40. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133: 1302–7.
41. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011; 472:57–63.
42. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–84.
43. Miller CA, Corbin KD, da Costa KA, Zhang S, Zhao X, Galanko JA, Blevins T, Bennett BJ, O'Connor A, Zeisel SH. Effect of egg ingestion on trimethylamine-N-oxide production in humans: a randomized, controlled, dose-response study. Am J Clin Nutr 2014;100:778–86.
44. Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr 2008;87:424–30.
45. Iribarren C, Folsom AR, Jacobs DR Jr., Gross MD, Belcher JD, Eckfeldt JH; ARIC Study Investigators. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis: a case-control study. Atherosclerosis Risk in Communities. Arterioscler Thromb Vasc Biol 1997; 17:1171–7.
46. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, et al. Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles Atherosclerosis Study. Circulation 2001;103:2922–7.
47. Kim JE, Leite JO, DeOgburn R, Smyth JA, Clark RM, Fernandez ML. A lutein-enriched diet prevents cholesterol accumulation and decreases oxidized LDL and inflammatory cytokines in the aorta of guinea pigs. J Nutr 2011;141:1458–63.
48. Wang MX, Jiao JH, Li ZY, Liu RR, Shi Q, Ma L. Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy nonsmokers. Atherosclerosis 2013;227:380–5.
49. National Resources Institute Finland. Balance sheet for food commodities. Version current June 30, 2015 [cited 2015 Aug 30]. Available from: http://stat.luke.fi/en/balance%20sheet%20for%20food%20commodities.
50. Valsta LM, Tapanainen H, Sundvall J, Laatikainen T, Mannisto S, Pietinen P, Vartiainen E. Explaining the 25-year decline of serum cholesterol by dietary changes and use of lipid-lowering medication in Finland. Public Health Nutr 2010;13:932–8.

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