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Associations of Dietary Cholesterol, Serum Cholesterol, and Egg Consumption With Overall and Cause-Specific Mortality: Systematic Review and Updated Meta-Analysis

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BACKGROUND: Despite substantial research highlighting the importance of exogenous dietary cholesterol intake and endogenous serum cholesterol level in human health, a thorough evaluation of the associations is lacking. Our study objective was to examine overall and cause-specific mortality in relation to dietary and serum cholesterol, as well as egg consumption, and conduct an updated meta-regression analysis of cohort studies.

METHODS: We conducted a prospective analysis of 27 078 men in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). Multivariable-controlled cause-specific Cox proportional hazards regression models were used to calculate hazard ratios and 31-year absolute mortality risk differences. A systematic review and meta-analysis of cohort studies was also performed (PROSPERO [URL: https://www.crd.york.ac.uk/prospero/; Unique identifier: CRD42021272756]).

RESULTS: Based on 482 316 person-years of follow-up, we identified 22 035 deaths, including 9110 deaths from cardiovascular disease (CVD). Greater dietary cholesterol and egg consumption were associated with increased risk of overall and CVD-related mortality. Hazard ratios for each additional 300 mg cholesterol intake per day were 1.10 and 1.13 for overall and CVD-related mortality, respectively; for each additional 50-g egg consumed daily, hazard ratios were 1.06 and 1.09, respectively, for overall and CVD-related mortality (all P values<0.0001). After multivariable adjustment, higher serum total cholesterol concentrations were associated with increased risk of CVD-related mortality (hazard ratios per 1 SD increment, 1.14; P<0.0001). The observed associations were generally similar across cohort subgroups. The updated meta-analysis of cohort studies on the basis of 49 risk estimates, 3 601 401 participants, and 255 479 events showed consumption of 1 additional 50-g egg daily was associated with significantly increased CVD risk (pooled relative risk, 1.04 [95% CI, 1.00–1.08]; I^2=80.1%). In the subgroup analysis of geographic regions ($P_{interaction}$=0.02), an increase of 50-g egg consumed daily was associated with a higher risk of CVD in US cohorts (pooled relative risk, 1.08 [95% CI, 1.02–1.14]) and appeared related to a higher CVD risk in European cohorts with borderline significance (pooled relative risk, 1.05), but was not associated with CVD risk in Asian cohorts.

CONCLUSIONS: In this prospective cohort study and updated meta-analysis, greater dietary cholesterol and egg consumption were associated with increased risk of overall and CVD-related mortality. Our findings support restricted consumption of dietary cholesterol as a means to improve long-term health and longevity.

Key Words: cholesterol, dietary ■ eggs, diet ■ meta-analysis ■ mortality ■ serum cholesterol ■ systematic review

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Clinical Perspective

What Is New?

- Whether dietary cholesterol and egg consumption influence risk of cardiovascular disease–related and overall mortality remains unclear.
- The current analysis, representing both an original cohort study of 27 078 Finnish men and an updated meta-analysis of 41 prospective cohort studies, demonstrates that participants with greater consumption of dietary cholesterol and eggs experienced increased risk of overall and cardiovascular disease–related mortality.
- In the updated meta-analysis, we found a significant positive association for egg consumption and cardiovascular disease risk in US cohorts, a marginal positive association in European cohorts, and no association in Asian cohorts.

What Are the Clinical Implications?

- These findings support restricted consumption of dietary cholesterol as a means to improve long-term cardiovascular health and longevity and provide compelling evidence relevant to dietary guidelines.

Nonstandard Abbreviations and Acronyms

ARD	absolute risk difference
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
BMI	body mass index
CVD	cardiovascular disease
HDL	high-density lipoprotein
HR	hazard ratio
RR	relative risk

Despite substantial research underscoring the importance of exogenous dietary cholesterol intake and endogenous serum cholesterol level in human health, a thorough, comprehensive examination of their associations with long-term health outcomes is not available. Dietary cholesterol is consumed in foods including eggs, beef, fish, and pork, whereas endogenous serum cholesterol is synthesized in the liver and extrahepatic tissues and circulates in the bloodstream.[1] Cholesterol plays an important role in cellular membrane structure and signal transduction and engages in essential regulatory functions including nutrient absorption, glucose metabolism, reproductive biology, and stress-related responses.[1,2] Laboratory evidence demonstrates that cholesterol can have cytotoxic activity (ie, that cellular cholesterol accumulation can induce membrane disruption, apoptosis, inflammation, and other stress-related responses).[2,3] Earlier experimental and observational studies highlighting the importance of cholesterol homeostasis for proper cellular and physiologic functions supported the hypothesis that impairment of cholesterol metabolism can be involved in the development of chronic diseases including cardiovascular disease (CVD) and cancer.[4–7]

As a result, longstanding dietary guidelines recommended a daily limit of 300 mg for dietary cholesterol intake to improve cardiovascular health.[8] However, the Scientific Report of the 2015 Dietary Guidelines Advisory Committee states that "cholesterol intake need not be limited because there is only a weak relationship between cholesterol intake and serum cholesterol concentrations," recommends that people "maintain minimum dietary cholesterol while consuming a healthy eating pattern," and states that "egg consumption should be considered part of a healthy diet."[9,10] A common and affordable food, eggs are one of the primary sources of dietary cholesterol, with 186 mg of cholesterol in a large boiled egg; however, they also contain a wide range of other high-quality nutrients, including protein, fatty acids, vitamins, and minerals.[11] The updated Scientific Report of the 2020 Dietary Guidelines Advisory Committee mentions that "it seems prudent to recommend lower intake of foods high in dietary cholesterol" and "the lack of studies evaluating a number of outcomes highlights the need for additional research [on dietary cholesterol]."[12]

For >2 decades, epidemiologic studies have evaluated the associations between higher dietary cholesterol and egg consumption and disease risk, with conflicting findings. Some studies demonstrate increased risk of CVD[6,7,13] and mortality[6,14–16]; others show null associations for CVD[16–18] and mortality[18–20] or inverse associations.[21,22] The reported risk estimates and directionality vary considerably, however, in part according to study design, number of events, source population, consumption levels, control for confounding, and length of follow-up, leaving the associations unclear.

In an effort to provide evidence from a more comprehensive assessment relevant to dietary guidelines and healthful dietary patterns, we examined the associations between dietary cholesterol intake, serum cholesterol level, and egg consumption and risk of overall and cause-specific mortality in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention) of 27 000 participants followed for >3 decades. On the basis of the findings, we performed an updated meta-analysis of the association between egg consumption and risk of CVD and CVD-related mortality that included the current study.

METHODS

Data Sharing

Because of previously enacted EU General Data Protection Regulation privacy rules and an existing data use agreement

between Finland and the US National Cancer Institute, the ATBC Study data and materials described in the article may not be made publicly available for the purposes of reproducing the findings. The principal investigators of the ATBC Study can be contacted with specific data requests (https://atbcstudy.cancer.gov/). To minimize the possibility of unintentionally sharing information that can be used to identify private information, a subset of the data generated for this study will be made available first on reasonable request.

Study Population

The ATBC Study was a controlled, 2×2 factorial primary prevention trial originally conducted to evaluate whether supplementation with α-tocopherol (50 mg/d), β-carotene (20 mg/d), or both could decrease cancer incidence. The ATBC Study recruited 29 133 Finnish male smokers between 1985 and 1988, age 50 to 69 years, from 14 study centers in southwest Finland. Intervention continued for 5 to 8 years until the end of intervention (April 30, 1993). Overnight fasting blood samples were collected and stored at −70°C and blood pressure, height, and weight were measured by skilled research nurses. The study was approved by institutional review boards at the US National Cancer Institute and the Finnish National Public Health Institute. All cohort participants provided written informed consent.

Exposure Assessment

Serum total cholesterol and high-density lipoprotein (HDL) concentrations were determined enzymatically (CHOD-PAP method; Boehringer Mannheim). Serum concentrations of α-tocopherol, β-carotene, and retinol were assayed by high-performance liquid chromatography.[23] Cohort members were invited to complete a food frequency questionnaire, which included dietary information on portion size and frequency of 276 food and beverage items in the past 12 months. In the food frequency questionnaire, participants were asked the following: (1) How often did you consume eggs, including boiled eggs, fried eggs, or omelets, in the past 12 months (1=more than once a day; 2=once a day; 3=nearly every day; 4=several times a week; 5=once a week; 6=once or several times a month; 7=rarely or never)? (2) How many eggs did you eat each time? A color picture booklet was distributed to all participants for assistance in portion size estimation. Daily nutrient intakes were calculated using the food composition database of the Finnish National Public Health Institute.[24] The validity and reproducibility of the food use questionnaire has been examined and reported, with intraclass correlation coefficients ranging from 0.6 to 0.7 for most dietary variables, including 0.66 and 0.58 for dietary cholesterol intake and egg consumption, respectively.[25] In the ATBC Study, 27 111 participants (93%) completed the food frequency questionnaire thoroughly for subsequent analysis. In the current study, 27 078 participants were retained in the final analytic cohort after excluding individuals with missing values for serum total cholesterol level (n=36) or dietary cholesterol or egg consumption (n=2022). The primary dietary sources of cholesterol intake (mean percentage of total daily dietary cholesterol) included eggs (43.6%), butter (13.2%), milk (8.2%), sausages (7.4%), fish (5.9%), pork (5.5%), cheese (3.3%), beef (2.9%), and other food items combined (10.1%).

Cohort Follow-Up and Mortality Assessment

Participants were followed from their study entry date in 1985 to 1988 until death or the end of follow-up (December 31, 2015), whichever came first. Vital status of participants was determined through linkage to the Causes of Death Registry, Statistics Finland (for details, see the Expanded Methods in the Supplemental Material).

Statistical Analysis

We used age-stratified, cause-specific Cox proportional hazards regression models (cause-specific hazard models to control for the competing risks) with attained person-time as the underlying time metric to determine hazard ratios (HRs) and corresponding 95% CIs for the associations between dietary cholesterol intake (per 300 mg/d), serum total cholesterol level (per 1 SD), or egg consumption (per 50 g/d) and risk of death, including overall and cause-specific mortality, respectively. For the latter analysis, mortality other than the outcome of interest was censored by the date of death. No violations were found for the tests of the proportional hazards assumption that modeled the interaction between intervals of follow-up observation time (categorial variable) and dietary cholesterol intake, serum total cholesterol level, or egg consumption (modeled linearly). Regarding the functional forms, a squared term of the given covariate (test for all continuous covariates) was included in the multivariable-adjusted model to check whether model fit was significantly improved or risk estimate was significantly changed. In these cases, we found that model fit and risk estimates did not change. The age-adjusted model included baseline age and total energy intake. Multivariable models were adjusted further for body mass index (BMI); cigarettes smoked per day; years of smoking; serum HDL cholesterol level; intervention assignment; systolic and diastolic blood pressure; history of CVD; history of diabetes; education; physical activity; serum concentrations of α-tocopherol, β-carotene, and retinol; alcohol intake; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Mutual adjustment was performed for dietary cholesterol intake and serum total cholesterol level. For egg consumption, regression models with or without dietary cholesterol were conducted separately. For each obtained HR from the primary models, adjusted absolute risk differences (ARDs) were computed for the tested exposure variables at the end of follow-up of 31 years. The corresponding 95% CIs for each ARD were estimated using 300 bootstrap samples.

We conducted stratified analyses of overall and CVD-related mortality on the basis of other exposure variables (for details, see the Expanded Methods in the Supplemental Material). We performed 9 further sensitivity analyses: (1) To control for residual confounding, we adjusted for propensity scores that reflected the associations between the primary exposure variables and the aforementioned potential confounding covariates.[26] (2) To decrease reverse causality bias, we excluded the first 2 and 5 years of follow-up. (3) To minimize bias from potential influence of preexisting illness on exposure variables, we excluded individuals with self-reported history of diabetes at baseline. (4) To test more parsimonious models, we adjusted for age; cigarettes smoked per day; years of smoking; intervention assignment; systolic and diastolic blood pressure; history of CVD; education; physical activity;

levels of serum α-tocopherol, β-carotene, and retinol; and daily dietary total energy and alcohol intake. (5) The distributions of dietary cholesterol, serum total cholesterol, and egg consumption were winsorized at the 0.5 and 99.5 percentiles before modeling. (6) To obtain risk estimates using different increment units, we evaluated for each additional 50 mg dietary cholesterol intake per day up to 600 mg/d, each additional 0.5 mmol/L (19.34 mg/dL) up to 3 mmol/L (116 mg/dL) serum total cholesterol level, and each additional 25 g of eggs consumed per day up to 200 g/d. (7) To estimate mortality risk for dietary cholesterol intake, we considered serum total cholesterol level and egg consumption using quintile categories. (8) To apply diet/nutrient density models, dietary cholesterol intake and egg consumption were regressed on total energy intake (grams per 1000 kcal) along with energy intake in the models. (9) To control for potential confounding from specific foods, we adjusted further for daily intakes of vegetables, fruit, legumes, whole grains, red and processed meat, fish, and potatoes (as quintile categories) in the models.

Although missing values for all covariables were <5% of the study population, a missing value indicator variable was generated for each before modeling. All analyses were conducted using SAS software, version 9.4 (SAS Institute). All reported *P* values are 2-sided at a type I error rate of 0.05. To control for multiple comparisons, the Bonferroni correction threshold was used to define statistical significance: 0.05/15=0.0033 for primary and secondary tests (5 tests for 3 examined exposure variables) and 0.05/14=0.0036 for the interaction tests in stratified subgroups.

Systematic Review and Meta-Analysis of Associations Between Egg Consumption and Risk of CVD and CVD-Related Mortality

We performed a systematic search and updated meta-analysis including the current data as well as previous prospective cohort analyses that examined the association of egg consumption with risk of CVD and CVD-related mortality in the general population. The meta-analysis was conducted on the basis of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis)[27] and the systematic review protocol was registered at the international prospective register (PROSPERO [URL: https://www.crd.york.ac.uk/prospero/; Unique identifier: CRD42021272756]). The systematic search was completed through 5 August 2021 of online databases, including Web of Science, PubMed, and Embase.

Two reviewers (L. Gan and Dr Huang) performed article searches independently on the basis of predefined criteria. Data were obtained from the eligible articles, including first author name, publication year, study population and cohort name, country where the cohort was conducted, sample size, follow-up duration, baseline age ranges of participants, approaches for dietary assessment, methods for outcome ascertainment, egg consumption categories, and adjustment for potential confounders. We extracted data for risk estimates and their 95% CIs from the fully adjusted models (for details, see the Expanded Methods in the Supplemental Material). In terms of egg consumption, we used the median value or the midpoint of the upper and lower bound of the intake category when data were available. If the upper bound was not reported for the highest category, the upper bound was estimated by multiplying the lower bound by 1.75. In the meta-analysis, we used lowest category of egg consumption as the reference group.[28] When the included studies did not present person-years for each consumption category, we imputed it on the basis of available data.[6,16,18,20,29–34] When the dose–response estimates were missing for a study, we computed the relevant estimates of relative risk (RR) on the basis of the trend of log-relative risk.[35]

We calculated the RR of CVD (including CVD-related mortality) associated with egg consumed per day (per 50 g/d) for each study and used the random-effects models of meta-analysis to compute the pooled relative risk estimate. Heterogeneity was examined using the Cochran Q test and the I^2 statistic. Each individual study was excluded from the overall meta-analysis individually and the RRs were recalculated to determine which, if any, studies drove the heterogeneity. Univariate meta-regression was conducted using study-level data to evaluate potential causes of heterogeneity. We conducted subgroup meta-analysis stratifying by sex, number of participants, duration of follow-up, number of events, geographical location, risk of bias, dietary assessment, as well as CVD outcome (incident CVD and CVD-related mortality).

Egger tests and funnel plots were computed to examine potential publication bias. We applied the Newcastle-Ottawa Scale to evaluate possible biases for the eligible studies. Stata version 16.0 was used to conduct statistical analyses for the meta-analysis.

RESULTS

Median daily dietary cholesterol intake and egg consumption in the ATBC Study were 538 mg (mean value, 582 mg) and 44.6 g (mean value, 53.3 g), respectively, and median serum total cholesterol level was 240 mg/dL (6.2 mmol/L). Participants with greater cholesterol intake were more likely to have lower serum vitamin E concentrations, less education, and lower prevalence of diabetes and CVD (Table 1 and Table S1; for details, see the Expanded Results in the Supplemental Material). Spearman correlation coefficients for dietary cholesterol intake, serum total cholesterol level, and egg consumption and a spectrum of dietary factors are presented in Table S2.

On the basis of 31 years of cohort follow-up, an average of 18.2 years and 482316 person-years, there were 22035 deaths, including 9110 from CVD (7450 heart disease and 1621 stroke) and 7213 from cancer. Each additional 300 mg cholesterol daily intake increment was significantly associated with increased risk of age-adjusted overall, CVD, heart disease, and cancer mortality, representing 8% to 10% higher risks. Adjustment for several other risk factors strengthened the cholesterol–mortality risk estimates somewhat, with HRs of 1.10, 1.13, and 1.13 for overall, CVD, and heart disease, respectively (all *P* values<0.0001). Corresponding adjusted ARDs (95% CIs) per each additional 300 mg cholesterol daily intake at 31 years of follow-up were of 1.80% (1.23%–2.39%), 1.83% (1.14%–2.48%), and 1.76% (1.19%–2.45%) for mortality overall, from

Table 1. Baseline Characteristics of Cohort Participants According to Quintile Categories of Dietary Cholesterol, Serum Total Cholesterol, and Egg Consumption in the ATBC Study*

	Dietary cholesterol (quintile)			Serum total cholesterol (quintile)			Egg consumption (quintile)		
Characteristics	1	3	5	1	3	5	1	3	5
Age, y	57.7 (5.2)	57.2 (5.1)	56.5 (4.8)	57.5 (5.2)	57.0 (5.0)	57.0 (4.9)	57.7 (5.2)	57.2 (5.0)	56.5 (4.8)
Cigarettes/d	19.5 (8.7)	20.4 (8.7)	21.8 (9.1)	20.6 (9.0)	20.2 (8.8)	20.4 (8.7)	20.1 (8.9)	20.2 (8.6)	21.4 (9.0)
Years of smoking	36.1 (8.8)	36.0 (8.3)	35.6 (8.3)	36.2 (8.7)	35.7 (8.4)	35.9 (8.3)	36.4 (8.7)	36.0 (8.3)	35.5 (8.3)
Systolic blood pressure, mm Hg	143 (26)	142 (20)	141 (19)	141 (23)	142 (23)	143 (19)	143 (26)	142 (19)	142 (19)
Diastolic blood pressure, mm Hg	88 (21)	87 (11)	88 (11)	87 (16)	88 (16)	88 (11)	88 (24)	87 (11)	88 (11)
Serum total cholesterol, mg/dL	239.3 (46.0)	241.2 (65.7)	242.0 (44.1)	181.7 (17.8)	239.7 (6.2)	305.4 (30.2)	243.6 (46.4)	239.7 (46.4)	239.7 (42.5)
Serum HDL cholesterol, mg/dL	47.6 (89.7)	46.8 (53.0)	47.2 (12.4)	46.4 (53.0)	46.0 (12.0)	47.6 (73.5)	45.6 (12.4)	46.0 (12.4)	46.8 (12.4)
Serum α-tocopherol, mg/L	12.4 (4.2)	11.9 (3.4)	11.6 (3.3)	9.3 (2.3)	11.8 (2.5)	15.0 (4.6)	12.1 (4.1)	11.9 (3.3)	11.9 (3.5)
Serum β-carotene, μg/L	211 (226)	212 (161)	210 (171)	167 (148)	212 (173)	255 (194)	219 (222)	211 (168)	201 (166)
Serum retinol, μg/L	588 (137)	586 (125)	591 (131)	546 (127)	591 (124)	627 (132)	586 (134)	587 (126)	593 (133)
BMI, kg/m^2	26.2 (4.2)	26.4 (4.4)	26.6 (4.4)	26.1 (4.6)	26.3 (3.8)	26.5 (3.9)	26.1 (4.6)	26.3 (3.8)	26.5 (3.9)
Education, % > elementary school	24.7	21.2	18.8	23.6	21.4	19.9	22.1	22.4	21.8
Physically active, %	19.4	22.7	20.9	19.6	21.1	21.5	19.8	21.6	20.6
History of CVD, %	47.6	39.7	38.0	42.2	41.3	41.3	46.9	40.4	39.3
History of diabetes, %	5.6	4.0	3.8	6.0	3.5	3.6	5.2	3.7	4.1
Daily dietary intake									
Energy, kcal	2005 (447)	2632 (488)	3485 (793)	2685 (764)	2694 (750)	2680 (738)	2267 (594)	2663 (646)	3173 (835)
Energy from saturated fatty acids, %	14.8 (4.1)	17.7 (4.1)	19.1 (4.1)	16.8 (4.4)	17.4 (4.3)	17.9 (4.4)	17.0 (4.9)	17.5 (4.2)	17.5 (4.1)
Alcohol, g	17.2 (22.5)	17.4 (20.6)	20.3 (22.7)	18.5 (22.9)	18.1 (21.6)	17.3 (21.1)	17.0 (22.0)	17.4 (20.5)	20.7 (22.8)
Fruit, g	106 (91)	126 (94)	155 (117)	131 (102)	130 (102)	126 (100)	109 (94)	127 (97)	149 (114)
Vegetables, g	100 (68)	113 (67)	131 (78)	115 (73)	114 (71)	112 (69)	100 (69)	113 (67)	127 (78)
Red meat, g	54.1 (23.9)	71.0 (30.6)	89.2 (41.6)	69.6 (33.6)	71.3 (33.8)	73 (34.6)	61.7 (30.9)	70.9 (32.0)	80.8 (38.1)
Alternate Mediterranean Diet Score	25.9 (5.1)	24.9 (5.0)	24.1 (5.0)	24.9 (5.2)	25.0 (5.0)	25.0 (5.1)	24.9 (5.3)	24.9 (5.0)	24.8 (5.0)

ATBC indicates Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BMI, body mass index; CVD, cardiovascular disease; and HDL, high-density lipoprotein.
*Values are mean (SD) or percentages as indicated.

CVD, and from heart disease, respectively. By contrast, the multivariable-adjusted positive association between cholesterol intake and cancer and stroke mortality did not reach the Bonferroni correction *P* value threshold of 0.0033 (Table 2).

Higher concentrations of serum total cholesterol were significantly inversely associated with age-adjusted mortality overall, as well as from stroke and from cancer, but positively associated with CVD and heart disease (Table 2). After adjustment for several potential confounding factors, however, the associations for overall and stroke mortality were attenuated and that with cancer mortality did not achieve the Bonferroni correction threshold. By contrast, multivariable adjustment strengthened the positive risk estimates for CVD-related and heart disease–related mortality, with the HRs (95% CIs) per 1 SD increment of serum total cholesterol level being 1.14 (1.11–1.17) and 1.16 (1.13–1.20), respectively (all *P* values<0.0001). At the time of follow-up through 31 years, the corresponding adjusted ARDs (95% CIs) per 1 SD increment were of 1.96% (1.57%–2.40%) and 2.09% (1.71%–2.51%) for mortality from CVD and heart disease, respectively (Table 2).

Consumption of 1 additional 50-g egg per day was associated with significantly increased age-adjusted overall, CVD-related, and heart disease–related mortality (Table 3). After multivariable adjustment (model 2), the respective risk estimates of 6%, 9%, and 9% remained statistically significant (all *P* values<0.0001), with corresponding adjusted ARDs of 1.19% (95% CI, 0.75%–1.65%), 1.25% (95% CI, 0.72%–1.74%), and 1.20% (95% CI, 0.69%–1.71%). After further adjustment for dietary cholesterol intake (model 3), the egg consumption associations with overall, CVD-related, and heart disease–related mortality were no longer significant; however, with HRs of 0.91, 0.92, and 0.91 (all *P* values>0.0033). Egg consumption was not related to stroke or cancer mortality (all *P* values>0.0033; Table 3).

Table 2. Associations Between Dietary Cholesterol, Serum Total Cholesterol, and Overall and Cause-Specific Mortality in the ATBC Study

	Dietary cholesterol (per 300 mg)*			Serum total cholesterol (per 1 SD)†		
Cause of death	ARD, % (95% CI)	HR (95% CI)	*P* value	ARD, % (95% CI)	HR (95% CI)	*P* value
All causes						
Age-adjusted	1.83 (1.41, 2.27)	1.09 (1.07, 1.12)	<0.0001	−0.48 (−0.79, −0.21)	0.98 (0.96, 0.99)	0.0008
Multivariable‡	1.80 (1.23, 2.39)	1.10 (1.06, 1.13)	<0.0001	0.35 (−0.085, 0.91)	1.02 (1.00, 1.04)	0.05
CVD						
Age-adjusted	1.48 (0.95, 1.99)	1.10 (1.06, 1.14)	<0.0001	1.26 (0.90, 1.59)	1.08 (1.06, 1.11)	<0.0001
Multivariable‡	1.83 (1.14, 2.48)	1.13 (1.08, 1.18)	<0.0001	1.96 (1.57, 2.40)	1.14 (1.11, 1.17)	<0.0001
Heart disease						
Age-adjusted	1.40 (0.87, 1.92)	1.10 (1.06, 1.14)	<0.0001	1.64 (1.29, 1.98)	1.12 (1.10, 1.15)	<0.0001
Multivariable‡	1.76 (1.19, 2.45)	1.13 (1.08, 1.19)	<0.0001	2.09 (1.71, 2.51)	1.16 (1.13, 1.20)	<0.0001
Stroke						
Age-adjusted	0.45 (−0.03, 0.88)	1.08 (0.99, 1.17)	0.069	−0.52 (−0.84, −0.22)	0.92 (0.87, 0.96)	0.0007
Multivariable‡	0.55 (−0.13, 1.13)	1.10 (0.99, 1.22)	0.088	0.17 (−0.26, 0.59)	1.03 (0.96, 1.10)	0.40
Cancer						
Age-adjusted	1.18 (0.62, 1.76)	1.08 (1.04, 1.13)	<0.0001	−0.79 (−1.19, −0.41)	0.95 (0.93, 0.97)	<0.0001
Multivariable‡	0.87 (0.17, 1.63)	1.06 (1.01, 1.12)	0.02	−0.59 (−1.12, −0.018)	0.96 (0.93, 0.99)	0.0083

ARD indicates absolute risk difference; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; CVD, cardiovascular disease; HDL, high-density lipoprotein; and HR, hazard ratio.

*ARDs and HRs of overall and cause-specific mortality are for each 300-mg increment of dietary cholesterol consumption per day.

†ARDs and HRs of CVD mortality are for 1-SD increment of serum total cholesterol.

‡Models adjusted for baseline age; body mass index; cigarettes smoked per day; years of smoking; serum HDL cholesterol; intervention assignment; systolic and diastolic blood pressure; history of CVD; diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; and daily dietary total energy, alcohol intake, and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Mutual adjustment was performed for dietary cholesterol and serum total cholesterol. Total event number for death from all causes, CVD, heart disease, stroke, or cancer is 22 035, 9110, 7450, 1621, and 7213, respectively.

Figure 1A through 1C and Figure 2A and 2B present cohort subgroup mortality findings for dietary cholesterol intake, serum total cholesterol level, and egg consumption. Overall, we observed similar risk estimates across strata of age; cigarettes per day; BMI; CVD history; trial intervention arms; diet quality; total energy intake; dietary cholesterol intake; saturated fatty acid intake; serum total cholesterol level; serum concentrations of α-tocopherol, β-carotene, and retinol; and years of follow-up (all *P* values>0.0036; Bonferroni correction threshold).

Propensity score adjustment did not materially change the risk estimates of dietary cholesterol intake, serum total cholesterol level, or egg consumption with overall or CVD-related mortality (all *P* values<0.0001; Table S3). All risk estimates remained essentially the same in the lag analyses (all *P* values<0.0001; Table S4). Excluding participants with self-reported diabetes history also did not alter the observed associations (all *P* values<0.0001; Table S5). Our findings remained essentially unchanged using parsimonious models (Table S6) or winsorized distributions of dietary cholesterol intake, serum total cholesterol level, and egg consumption at the 0.5th and 99.5th percentiles (Table S7). The multivariable-adjusted HRs and corresponding 95% CIs of overall and CVD-related mortality according to gradual increment units for dietary cholesterol intake, serum total cholesterol level, and egg consumption are presented in Tables S8 through S10. Our findings remained largely unchanged according to the quintiles of exposure variables (P_{trend}≤0.003; Table S11). Quintile models for dietary cholesterol and egg consumption were further adjusted for energy intake using the nutrient density method and our findings were not materially altered (P_{trend}≤0.0002; Table S12). Our findings remained essentially unchanged after further adjustment for specific foods including vegetables, fruit, legumes, whole grains, red and processed meat, fish, and potatoes (Table S13; for details, see the Expanded Results in the Supplemental Material).

Systematic Review and Meta-Analysis

Overall, our initial search included 1036 articles, and 40 studies (41 including the current study) met the predefined inclusion criteria and were kept in the final meta-analysis (Figure S1 and Tables S14 and S15). The covariates in the multivariable models of each individual study are presented in Tables S15 and S16. For the Newcastle-Ottawa Scale assessment, 17 studies (20 risk estimates) received a score ≥7, which suggested low risk of bias (Table S17).

The meta-analysis for the association of daily egg consumption and risk of CVD (including CVD-related

Table 3. Associations Between Daily Egg Consumption and Overall and Cause-Specific Mortality in the ATBC Study

Cause of death	Egg consumption (per 50 g/d)*		
	ARD, % (95% CI)	HR (95% CI)	*P* value
All causes			
Model 1: Age-adjusted	1.18 (0.81, 1.56)	1.06 (1.04, 1.08)	<0.0001
Model 2: Multivariable†	1.19 (0.75, 1.65)	1.06 (1.04, 1.09)	<0.0001
Model 3: Multivariable‡	−1.83 (−3.50, −0.14)	0.91 (0.84, 0.99)	0.029
CVD			
Model 1: Age-adjusted	1.00 (0.55, 1.44)	1.07 (1.03, 1.10)	<0.0001
Model 2: Multivariable†	1.25 (0.72, 1.74)	1.09 (1.05, 1.12)	<0.0001
Model 3: Multivariable‡	−1.33 (−3.51, 0.62)	0.92 (0.81, 1.04)	0.18
Heart disease			
Model 1: Age-adjusted	0.94 (0.49, 1.38)	1.07 (1.03, 1.10)	<0.0001
Model 2: Multivariable†	1.20 (0.69, 1.71)	1.09 (1.05, 1.13)	<0.0001
Model 3: Multivariable‡	−1.34 (−3.57, 0.70)	0.91 (0.79, 1.05)	0.19
Stroke			
Model 1: Age-adjusted	0.32 (−0.11, 0.70)	1.06 (0.98, 1.13)	0.13
Model 2: Multivariable†	0.37 (−0.15, 0.80)	1.06 (0.98, 1.15)	0.13
Model 3: Multivariable‡	−0.46 (−2.50, 1.41)	0.93 (0.68, 1.25)	0.62
Cancer			
Model 1: Age-adjusted	0.58 (0.087, 1.10)	1.04 (1.01, 1.08)	0.021
Model 2: Multivariable†	0.56 (0.022, 1.16)	1.04 (1.00, 1.08)	0.047
Model 3: Multivariable‡	−1.04 (−3.10, 1.12)	0.93 (0.81, 1.08)	0.34

ARD indicates absolute risk difference; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; CVD, cardiovascular disease; HDL, high-density lipoprotein; and HR, hazard ratio.

*ARDs and HRs of overall and cause-specific mortality are for each 50-g increment of egg consumption per day.

†Models were adjusted for age; body mass index; cigarettes smoked per day; years of smoking; serum total and HDL cholesterol; intervention assignment; systolic and diastolic blood pressure; history of CVD; diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; and daily dietary total energy, alcohol intake, and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Total event number for death from all causes, CVD, heart disease, stroke, or cancer is 22 035, 9110, 7450, 1621, and 7213, respectively.

‡Models were further adjusted for dietary cholesterol.

mortality) contained 49 risk estimates, 3 601 401 participants, and 255 479 events (Figure 3). In the pooled RR of the meta-analysis, consumption of 1 additional 50-g egg per day was associated with significantly increased risk of CVD (pooled RR, 1.04 [95% CI, 1.00–1.08]; Figure 3). We did not observe any evidence of publication bias for the association between egg consumption and CVD risk (Figure S2). However, evidence of substantial heterogeneity existed between studies (I^2=80.1%; Figure 3). On the other hand, no individual study disproportionately influenced the heterogeneity alone (Figure S3). There were no statistically significant interactions in the predefined subgroup meta-analyses (Table S18 and Figure S4), with the exception of geographical region (United States, Europe, and Asia; Figure S5), with $P_{interaction}$=0.02. An increase of 50 g of egg consumed per day was associated with a higher risk of CVD among cohorts in the United States (RR, 1.08 [95% CI, 1.02–1.14]) and appeared to be related to a higher CVD risk in European cohorts, with borderline significance (RR, 1.05 [95% CI, 0.98–1.14]), but was not associated with CVD risk in Asian cohorts (RR, 0.96 [95% CI, 0.87–1.06]).

DISCUSSION

In this large cohort of 27 078 Finnish men followed for up to 31 years, participants with greater consumption of dietary cholesterol and eggs experienced modest but significant increases in risk of overall, CVD-related, and heart disease–related mortality independent of other risk factors including serum total cholesterol level. The increased risk of mortality was similar across cohort subgroups. The observed egg–mortality associations were diminished after adjustment for dietary cholesterol intake. Findings from the updated meta-regression analysis provided strong support for the overall positive association between egg consumption (eg, 1 egg per day) and risk of CVD, including CVD-related mortality. We observed some evidence of heterogeneity in the association across the studies (I^2=80.1%), however,

with population geographical location being associated with study differences in the United States, Europe, and Asia. Whereas there was a significant positive association between egg consumption (50 g/d) and CVD risk in the US cohorts, we found a marginal positive association in the European cohorts and no association in the Asian cohorts. There were fewer participants in the Asian cohorts (n=921 563) than in the US cohorts (n=1 388 758), which may have limited power to examine the associations. In addition, whether the egg–mortality associations are modified by cooking practices (eg, boiling versus frying) may need further investigation. The other predefined subgroup meta-analyses revealed stable positive egg–CVD risk associations, including for sex, cohort size, duration of follow-up, number of events, risk of study bias, and method of dietary assessment.

A recent pooled analysis of 3 US cohorts including the Nurses' Health Study, the Nurses' Health Study II, and the Health Professionals' Follow-Up Study found that moderate egg consumption (<1 egg per day) was

A

Subgroup	Absolute risk difference, % (95% CI)	Hazard ratio (95% CI)	$P_{interaction}$
Age at baseline, y			
<57	2.85 (1.91, 3.90)	1.13 (1.08, 1.18)	0.074
≥57	1.01 (3.04, 1.70)	1.06 (1.02, 1.10)	
Cigarettes smoked per day			
<16	1.47 (0.52, 2.50)	1.08 (1.02, 1.14)	0.88
16 to <20	0.83 (-0.19, 1.83)	1.04 (0.99, 1.10)	
≥20	2.85 (1.81, 3.83)	1.14 (1.09, 1.20)	
BMI (kg/m^2)			
<30	1.62 (0.99, 2.26)	1.09 (1.05, 1.12)	0.62
≥30	2.07 (0.75, 3.45)	1.10 (1.03, 1.18)	
History of cardiovascular disease			
No	1.79 (1.07, 2.55)	1.10 (1.06, 1.14)	0.31
Yes	1.70 (0.78, 2.60)	1.09 (1.04, 1.13)	
Trial intervention arm			
Alpha-tocopherol	1.45 (0.62, 2.26)	1.08 (1.03, 1.12)	0.091
No alpha-tocopherol	2.17 (1.37, 3.00)	1.12 (1.07, 1.16)	
Beta-carotene	2.54 (1.79, 3.36)	1.14 (1.09, 1.18)	0.29
No beta-carotene	0.99 (0.12, 1.80)	1.05 (1.01, 1.10)	
Eating a high-quality diet			
No	1.54 (0.89, 2.14)	1.08 (1.05, 1.12)	0.38
Yes	2.78 (1.49, 4.20)	1.15 (1.08, 1.22)	
Total energy intake			
Low	2.49 (1.52, 3.45)	1.14 (1.08, 1.19)	0.78
High	1.43 (0.67, 2.18)	1.07 (1.03, 1.11)	
Saturated fatty acids intake			
Low or medium	2.02 (1.31, 2.75)	1.11 (1.07, 1.14)	0.52
High	0.93 (-0.13, 2.00)	1.05 (0.99, 1.11)	
Serum total cholesterol, mg/dL			
<205	1.93 (1.17, 2.65)	1.11 (1.06, 1.15)	0.52
≥205	1.65 (0.73, 2.59)	1.08 (1.04, 1.13)	
Serum alpha-tocopherol, mg/L			
<11.5	1.87 (1.08, 2.64)	1.10 (1.06, 1.15)	0.49
≥11.5	1.64 (0.81, 2.47)	1.09 (1.04, 1.13)	
Serum beta-carotene, ug/L			
<172	2.21 (1.41, 3.02)	1.12 (1.08, 1.16)	0.60
≥172	1.20 (3.83, 2.01)	1.06 (1.02, 1.11)	
Serum retinol, ug/L			
<577	1.97 (1.17, 2.77)	1.11 (1.06, 1.15)	0.21
≥577	1.55 (0.72, 2.35)	1.08 (1.04, 1.12)	
Years of follow-up			
0-13	1.25 (0.63, 1.87)	1.09 (1.05, 1.12)	0.78
13 to <23	1.48 (0.80, 2.17)	1.10 (1.06, 1.14)	
≥23	1.82 (0.50, 3.15)	1.10 (1.05, 1.15)	

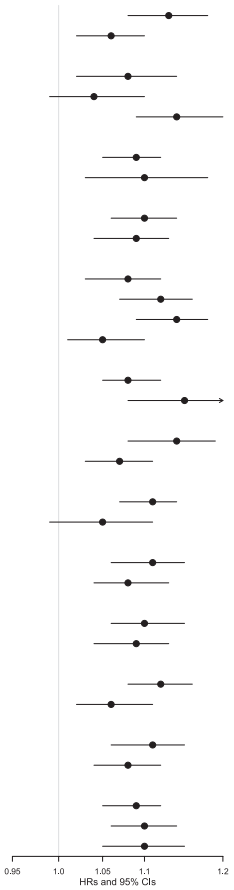
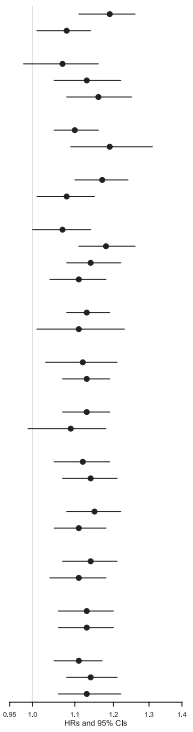


Figure 1. Association between cholesterol and mortality by selected risk factors in the ATBC Study.
A, Association between dietary cholesterol intake (per day) and overall mortality by selected risk factors in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). Absolute risk differences and hazard ratios (HRs) of overall mortality are for each 300-mg increment of dietary cholesterol consumption per day. Models were adjusted for age; body mass index (BMI); cigarettes smoked per day; years of smoking; serum total and high-density lipoprotein (HDL) cholesterol; intervention assignment; systolic and diastolic blood pressure; history of cardiovascular disease (CVD); diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; dietary intake of energy and alcohol; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. **B**, Association between dietary cholesterol intake (per day) and CVD mortality by selected risk factors in the ATBC Study. Absolute risk differences and HRs of CVD-related mortality are for each 300-mg increment of dietary cholesterol consumption per day. Models were adjusted for age; BMI; cigarettes smoked per day; years of smoking; serum total and HDL cholesterol; intervention assignment; systolic and diastolic blood pressure; history of CVD; diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; dietary intake of energy and alcohol; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. **C**, Association between serum total cholesterol level (per 1 SD) and CVD mortality by selected risk factors in the ATBC Study. Absolute risk differences and HRs of CVD-related mortality are for 1-SD increment of serum total cholesterol level. Models were adjusted for age; BMI; cigarettes smoked per day; years of smoking; serum HDL cholesterol; intervention assignment; systolic and diastolic blood pressure; history of CVD; diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; dietary intake of energy and alcohol; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. (*Continued*)

B

Subgroup	Absolute risk difference, % (95% CI)	Hazard ratio (95% CI)	$P_{interaction}$
Age at baseline, y			
<57	3.07 (1.89, 4.23)	1.19 (1.11, 1.26)	0.09
≥57	0.94 (0.11, 1.72)	1.08 (1.01, 1.14)	
Cigarettes smoked per day			
<16	0.97 (-0.27, 2.23)	1.07 (0.98, 1.16)	0.77
16 to <20	1.90 (0.73, 2.95)	1.13 (1.05, 1.22)	
≥20	2.38 (1.10, 3.55)	1.16 (1.08, 1.25)	
BMI (kg/m^2)			
<30	1.48 (0.68, 2.22)	1.10 (1.05, 1.16)	0.28
≥30	2.90 (1.36, 4.45)	1.19 (1.09, 1.31)	
History of cardiovascular disease			
No	2.31 (1.44, 3.16)	1.17 (1.10, 1.24)	0.039
Yes	1.27 (0.18, 2.29)	1.08 (1.01, 1.15)	
Trial intervention arm			
Alpha-tocopherol	1.05 (0.029, 2.00)	1.07 (1.00, 1.14)	0.17
No alpha-tocopherol	2.54 (1.64, 3.40)	1.18 (1.11, 1.26)	
Beta-carotene	2.05 (1.20, 2.97)	1.14 (1.08, 1.22)	0.58
No beta-carotene	1.54 (0.51, 2.42)	1.11 (1.04, 1.18)	
Eating a high-quality diet			
No	1.89 (1.13, 2.55)	1.13 (1.08, 1.19)	0.86
Yes	1.71 (0.15, 3.42)	1.11 (1.01, 1.23)	
Total energy intake			
Low	1.59 (0.48, 2.58)	1.12 (1.03, 1.21)	0.11
High	1.89 (1.00, 2.73)	1.13 (1.07, 1.19)	
Saturated fatty acids intake			
Low or medium	1.89 (1.04, 2.72)	1.13 (1.07, 1.19)	0.35
High	1.22 (-0.068, 2.44)	1.09 (0.99, 1.18)	
Serum total cholesterol, mg/dL			
<205	1.65 (0.73, 2.53)	1.12 (1.05, 1.19)	0.64
≥205	2.05 (0.99, 3.00)	1.14 (1.07, 1.21)	
Serum alpha-tocopherol, mg/L			
<11.5	2.05 (1.06, 3.00)	1.15 (1.08, 1.22)	0.27
≥11.5	1.60 (0.62, 2.46)	1.11 (1.05, 1.18)	
Serum beta-carotene, ug/L			
<172	1.97 (1.06, 2.80)	1.14 (1.07, 1.21)	0.75
≥172	1.54 (0.53, 2.53)	1.11 (1.04, 1.18)	
Serum retinol, ug/L			
<577	1.76 (0.79, 2.68)	1.13 (1.06, 1.20)	0.53
≥577	1.85 (0.86, 2.70)	1.13 (1.06, 1.20)	
Years of follow-up			
0-13	0.75 (0.22, 1.28)	1.11 (1.05, 1.17)	0.53
13 to <23	1.57 (0.94, 2.23)	1.14 (1.08, 1.21)	
≥23	1.65 (0.18, 3.06)	1.13 (1.06, 1.22)	



C

Subgroup	Absolute risk difference, % (95% CI)	Hazard ratio (95% CI)	$P_{interaction}$
Age at baseline, y			
<57	2.59 (1.84, 3.39)	1.16 (1.11, 1.20)	0.54
≥57	1.46 (1.07, 1.92)	1.12 (1.08, 1.16)	
Cigarettes smoked per day			
<16	1.70 (0.94, 2.36)	1.12 (1.07, 1.17)	0.84
16 to <20	2.15 (1.43, 2.84)	1.15 (1.10, 1.21)	
≥20	2.41 (1.57, 3.17)	1.17 (1.11, 1.22)	
BMI (kg/m^2)			
<30	1.94 (1.48, 2.41)	1.14 (1.11, 1.17)	0.87
≥30	2.17 (1.22, 3.25)	1.14 (1.07, 1.21)	
History of cardiovascular disease			
No	2.14 (1.56, 2.76)	1.16 (1.11, 1.20)	0.52
Yes	1.93 (1.35, 2.59)	1.12 (1.08, 1.16)	
Trial intervention arm			
Alpha-tocopherol	1.77 (1.16, 2.44)	1.12 (1.08, 1.17)	0.73
No alpha-tocopherol	2.14 (1.56, 2.76)	1.15 (1.11, 1.20)	
Beta-carotene	2.01 (1.47, 2.65)	1.14 (1.10, 1.19)	0.14
No beta-carotene	1.81 (1.23, 2.48)	1.13 (1.08, 1.17)	
Eating a high-quality diet			
No	1.91 (1.40, 2.40)	1.14 (1.10, 1.17)	0.97
Yes	2.15 (1.35, 3.01)	1.15 (1.09, 1.21)	
Total energy intake			
Low	1.73 (1.21, 2.27)	1.13 (1.09, 1.17)	0.71
High	2.22 (1.53, 2.81)	1.15 (1.11, 1.20)	
Saturated fatty acids intake			
Low or medium	2.10 (1.61, 2.60)	1.15 (1.11, 1.18)	0.96
High	1.56 (0.70, 2.43)	1.11 (1.05, 1.18)	
Dietary cholesterol, mg			
<538	1.92 (0.76, 2.83)	1.11 (1.07, 1.15)	0.022
≥538	1.93 (1.30, 2.59)	1.18 (1.13, 1.22)	
Serum alpha-tocopherol, mg/L			
<11.5	2.22 (1.50, 2.84)	1.16 (1.11, 1.22)	0.62
≥11.5	1.82 (1.28, 2.38)	1.13 (1.09, 1.17)	
Serum beta-carotene, ug/L			
<172	2.29 (1.70, 2.80)	1.17 (1.12, 1.21)	0.05
≥172	1.52 (0.93, 2.26)	1.11 (1.06, 1.15)	
Serum retinol, ug/L			
<577	2.17 (1.58, 2.71)	1.16 (1.12, 1.21)	0.90
≥577	1.70 (1.12, 2.27)	1.12 (1.08, 1.16)	
Years of follow-up			
0-13	1.21 (0.97, 1.49)	1.18 (1.14, 1.22)	0.034
13 to <23	1.15 (0.72, 1.57)	1.12 (1.08, 1.16)	
≥23	1.35 (0.44, 2.24)	1.11 (1.05, 1.16)	

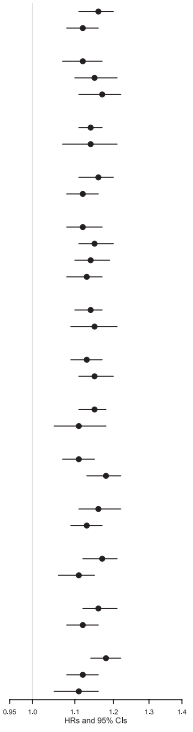


Figure 1 Continued.

not associated with CVD risk, even with adjustment for history of hypercholesterolemia and use of lipid-lowering medications.[17] A large analysis from the Prospective Urban Rural Epidemiology study also showed null associations for egg consumption and mortality.[18] The China Kadoorie Biobank Study, a large Asian cohort with nearly

0.5 million adults, demonstrated an inverse association between moderate consumption of egg and risk of CVD, with an HR of 0.89 (95% CI, 0.87–0.92) for daily egg consumers compared with nonconsumers.[22] The NIPPON DATA90 Study of >4000 Japanese women reported that those who consumed >2 eggs per day had increased overall mortality when compared with those consuming <1 egg per week, but showed no association for CVD-related mortality.[15] With respect to dietary cholesterol intake and CVD risk, a recent meta-analysis did not demonstrate a conclusive association owing to sparse data and heterogeneity across the available studies.[36] By contrast, a recent study of 30 000 participants from 6 US cohorts followed for up to 31 years showed dose–response associations such that each additional 300 mg of cholesterol intake daily was associated with 17% and 18% increased risk for CVD and overall mortality and that eating half an egg more per day was associated with 6% and 8% increased risk of CVD and overall mortality, respectively.[6] The sample sizes in the study by Zhong et al.[6] and our study were similar, although the former included a more diverse population, comprising 55% women and 31% Black participants. Although the dietary cholesterol–mortality risk estimates were slightly higher than in the current findings, the results of the 2 studies are essentially consistent, showing that egg–mortality associations were attenuated after adjusting for dietary cholesterol intake. Both studies used competing risk

A

Subgroup	Absolute risk difference, % (95% CI)	Hazard ratio (95% CI)	$P_{interaction}$
Age at baseline, y			
<57	1.79 (1.05, 2.59)	1.08 (1.05, 1.11)	0.15
≥57	0.73 (0.20, 1.25)	1.04 (1.01, 1.07)	
Cigarettes smoked per day			
<16	0.93 (0.19, 1.72)	1.05 (1.01, 1.10)	0.66
16 to <20	0.50 (-0.25, 1.28)	1.03 (0.99, 1.07)	
≥20	1.96 (1.16, 2.72)	1.10 (1.06, 1.13)	
BMI (kg/m^2)			
<30	1.08 (0.60, 1.57)	1.06 (1.03, 1.08)	0.90
≥30	1.31 (0.26, 2.36)	1.06 (1.01, 1.12)	
History of cardiovascular disease			
No	1.17 (0.62, 1.75)	1.06 (1.03, 1.09)	0.71
Yes	1.15 (0.43, 1.85)	1.06 (1.02, 1.09)	
Trial intervention arm			
Alpha-tocopherol	1.01 (0.39, 1.62)	1.05 (1.02, 1.08)	0.087
No alpha-tocopherol	1.39 (0.75, 2.05)	1.07 (1.04, 1.11)	
Beta-carotene	1.69 (1.10, 2.33)	1.09 (1.06, 1.12)	0.22
No beta-carotene	0.65 (-0.007, 1.26)	1.03 (1.00, 1.07)	
Eating a high-quality diet			
No	1.00 (0.49, 1.47)	1.05 (1.03, 1.08)	0.46
Yes	1.70 (0.71, 2.78)	1.09 (1.04, 1.14)	
Total energy intake			
Low	1.58 (0.85, 2.31)	1.09 (1.05, 1.13)	0.72
High	0.95 (0.37, 1.52)	1.05 (1.02, 1.08)	
Saturated fatty acids intake			
Low or medium	1.34 (0.80, 1.90)	1.07 (1.04, 1.10)	0.46
High	0.53 (-0.30, 1.38)	1.03 (0.98, 1.07)	
Serum total cholesterol, mg/dL			
<205	1.34 (0.75, 1.90)	1.07 (1.04, 1.10)	0.87
≥205	1.03 (0.33, 1.75)	1.05 (1.02, 1.09)	
Serum alpha-tocopherol, mg/L			
<11.5	1.22 (0.62, 1.82)	1.06 (1.03, 1.10)	0.86
≥11.5	1.07 (0.43, 1.71)	1.06 (1.02, 1.09)	
Serum beta-carotene, ug/L			
<172	1.48 (0.86, 2.10)	1.08 (1.05, 1.11)	0.46
≥172	0.76 (0.14, 1.37)	1.04 (1.01, 1.07)	
Serum retinol, ug/L			
<577	1.35 (0.76, 1.95)	1.07 (1.04, 1.11)	0.12
≥577	0.97 (0.33, 1.60)	1.05 (1.02, 1.08)	
Years of follow-up			
0-13	0.85 (0.37, 1.33)	1.06 (1.03, 1.09)	0.98
13 to <23	0.91 (0.38, 1.43)	1.06 (1.03, 1.09)	
≥23	1.30 (0.29, 2.34)	1.06 (1.02, 1.11)	

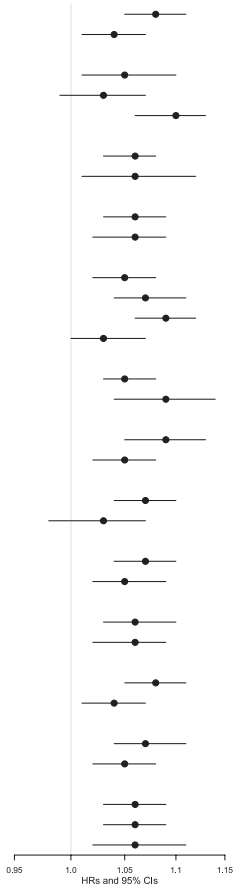


Figure 2. Association between egg consumption and mortality by selected risk factors in the ATBC Study.
A, Association between egg consumption (per day) and overall mortality by selected risk factors in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). Absolute risk differences and hazard ratios (HRs) of overall mortality are for each 50-g increment of egg consumption per day. Models were adjusted for age; body mass index; cigarettes smoked per day; years of smoking; serum total and high-density lipoprotein (HDL) cholesterol; intervention assignment; systolic and diastolic blood pressure; history of cardiovascular disease (CVD); diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; dietary intake of energy and alcohol; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. **B**, Association between egg consumption (per day) and CVD mortality by selected risk factors in the ATBC Study. Absolute risk differences and HRs of CVD-related mortality are for each 50-g increment of egg consumption per day. Models were adjusted for age; body mass index; cigarettes smoked per day; years of smoking; serum total and HDL cholesterol; intervention assignment; systolic and diastolic blood pressure; history of CVD; diabetes; education; physical activity; levels of serum α-tocopherol, β-carotene, and retinol; dietary intake of energy and alcohol; and percentage of energy from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. (*Continued*)

B

Subgroup	Absolute risk difference, % (95% CI)	Hazard ratio (95% CI)	$P_{interaction}$
Age at baseline, y			
<57	2.06 (1.16, 2.94)	1.12 (1.07, 1.18)	0.09
≥57	0.64 (0.0032, 1.21)	1.05 (1.00, 1.10)	
Cigarettes smoked per day			
<16	0.62 (-0.35, 1.59)	1.04 (0.98, 1.11)	0.69
16 to <20	1.26 (0.37, 2.06)	1.09 (1.03, 1.15)	
≥20	1.69 (0.68, 2.59)	1.11 (1.05, 1.18)	
BMI (kg/m^2)			
<30	0.99 (0.38, 1.55)	1.07 (1.03, 1.11)	0.085
≥30	1.99 (0.79, 3.17)	1.13 (1.05, 1.21)	
History of cardiovascular disease			
No	1.65 (0.99, 2.27)	1.12 (1.07, 1.17)	0.059
Yes	0.79 (-0.056, 1.57)	1.05 (1.00, 1.10)	
Trial intervention arm			
Alpha-tocopherol	0.75 (-0.036, 1.45)	1.05 (1.00, 1.10)	0.10
No alpha-tocopherol	1.70 (0.98, 2.37)	1.12 (1.07, 1.17)	
Beta-carotene	1.35 (0.69, 2.04)	1.09 (1.04, 1.14)	0.64
No beta-carotene	1.10 (0.32, 1.76)	1.07 (1.02, 1.13)	
Eating a high-quality diet			
No	1.29 (0.71, 1.79)	1.09 (1.05, 1.13)	0.54
Yes	0.97 (-0.24, 2.25)	1.06 (0.99, 1.15)	
Total energy intake			
Low	1.05 (0.20, 1.81)	1.08 (1.02, 1.14)	0.21
High	1.30 (0.62, 1.93)	1.09 (1.04, 1.13)	
Saturated fatty acids intake			
Low or medium	1.32 (0.68, 1.94)	1.09 (1.05, 1.13)	0.42
High	0.72 (-0.26, 1.64)	1.05 (0.98, 1.12)	
Serum total cholesterol, mg/dL			
<205	1.12 (0.41, 1.79)	1.08 (1.03, 1.13)	0.46
≥205	1.42 (0.60, 2.14)	1.10 (1.05, 1.15)	
Serum alpha-tocopherol, mg/L			
<11.5	1.35 (0.59, 2.06)	1.09 (1.04, 1.15)	0.57
≥11.5	1.13 (0.37, 1.78)	1.08 (1.03, 1.13)	
Serum beta-carotene, ug/L			
<172	1.35 (0.65, 1.97)	1.09 (1.04, 1.14)	0.49
≥172	1.03 (0.27, 1.76)	1.07 (1.02, 1.13)	
Serum retinol, ug/L			
<577	1.21 (0.49, 1.88)	1.09 (1.04, 1.14)	0.55
≥577	1.26 (0.48, 1.91)	1.08 (1.03, 1.14)	
Years of follow-up			
0-13	0.48 (0.069, 0.87)	1.07 (1.02, 1.12)	0.51
13 to <23	1.13 (0.65, 1.63)	1.11 (1.05, 1.16)	
≥23	1.14 (0.038, 2.20)	1.08 (1.01, 1.16)	

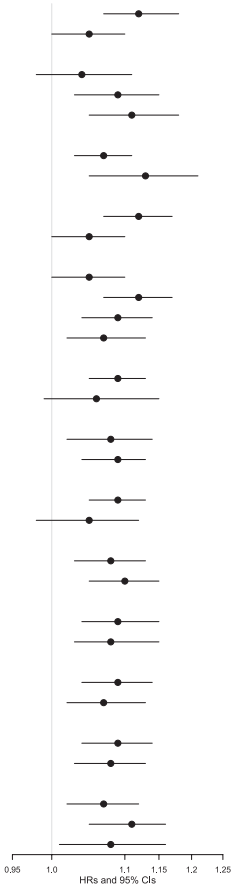


Figure 2 Continued.

models (ie, cause-specific hazard models). The dietary cholesterol–mortality risk estimates were higher among women than men in the study by Zhong and colleagues[6] (HR, 1.28 and 1.14 for women and men, respectively; $P_{interaction}$=0.02),[6] with the risk estimate among men being similar to that in our study. It should be noted, however, that the average daily cholesterol intake of 582 mg and egg consumption of 53.3 g were relatively high in our cohort as compared with corresponding values of 293 mg and 25.5 g in the general US population on the basis of the latest released data from the National Health and Nutrition Examination Survey.[37] Thus, our data provide mortality risk estimates for relatively higher consumption levels, which may partially account for some differences from the earlier studies in addition to population characteristics and length of follow-up. For example, average weekly egg consumption was 2.9 eggs in the Nurses' Health Study, 1.3 eggs in the Nurses' Health Study II, 2.4 eggs in the Health Professionals' Follow-Up Study, 3.3 eggs in the China Kadoorie Biobank Study, 3.9 eggs in the Prospective Urban Rural Epidemiology study, and 7.5 eggs in our 1980s cohort. Cholesterol is contained in the yolk of the egg,[38] whereas egg whites contain primarily proteins (ie, albumin).[39] Our findings, as well as those from other groups, show attenuation of the egg consumption–mortality association after adjusting for dietary cholesterol intake, supporting the hypothesis that it is the increased cholesterol intake from eggs that plays a key role and accounts for the elevated mortality associations. Additional investigations are warranted to explore the likely different biological roles of egg yolks versus egg whites and whole eggs.

A recent meta-analysis of 28 studies showed that there was no significant association between moderate egg consumption (ie, 1 egg per day) and risk of CVD.[17] The consistent results between that meta-analysis and our updated analysis include the considerable heterogeneity across European, US, and Asian cohorts. The previous meta-analysis suggested that there was a possible moderately increased risk of CVD of up to 19% among European cohorts (pooled RR, 1.05 [95% CI, 0.92–1.19])[17] and we observed a borderline significant positive association among European cohorts (pooled RR, 1.05 [95% CI, 0.98–1.14]), along with an 8% increased risk of CVD with moderate egg consumption (1 egg per day) in the US cohorts and no associa-

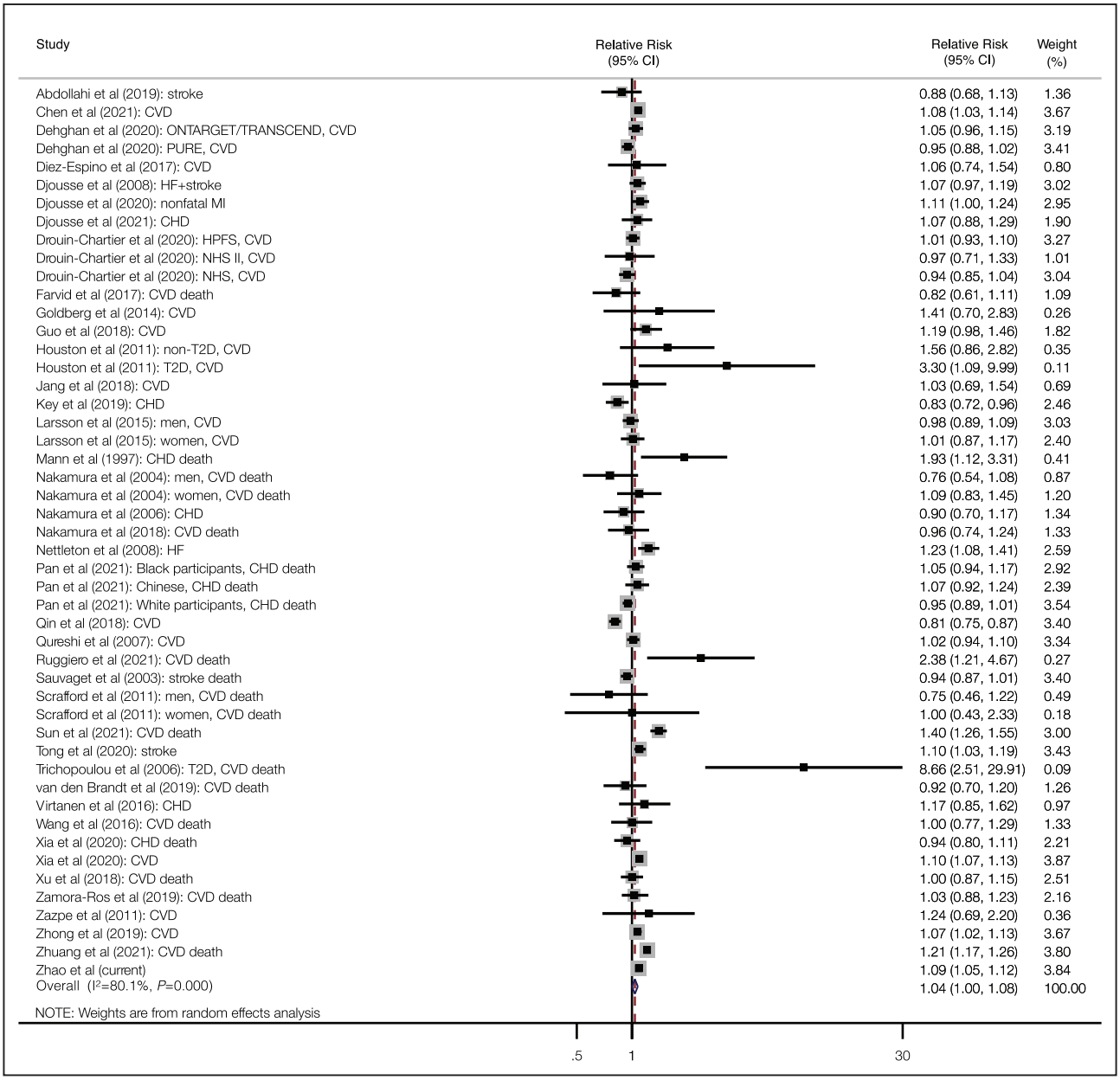


Figure 3. Association of egg consumption with cardiovascular disease risk for 1 egg per day increase using random-effects meta-analysis.

Squares reflect study-specific relative risk. Gray square areas are proportional to the individual study weight for the overall meta-analysis. Horizontal lines denote 95% CIs. I^2 refers to the proportion of heterogeneity among studies. CHD indicates coronary heart disease; CVD, cardiovascular disease; HF, heart failure; HPFS, Health Professionals' Follow-Up Study; MI, myocardial infarction; NHS, Nurses' Health Study; PURE, Prospective Urban Rural Epidemiology; and T2D, type 2 diabetes.

tion in Asian cohorts (pooled RR, 0.96). The significant overall positive association between moderate egg consumption and CVD risk in our updated meta-analysis remained stable among subgroup analyses, including when restricted to studies of low potential bias on the basis of Newcastle-Ottawa Score ≥7 (pooled RR, 1.04 [95% CI, 1.01–1.08]).

The association between dietary cholesterol intake and CVD-related mortality has been debated for decades but remains biologically plausible. Although dietary cholesterol intake and serum cholesterol level are only weakly associated, laboratory studies provide evidence that dietary cholesterol may be related to postprandial inflammation, oxidative stress–associated responses, and impairment of endothelial function.[40,41] In vivo studies have found that high dietary cholesterol intake can lead to an increased serum biomarker of chronic systemic inflammation, serum amyloid A,[42] which has been shown to be strongly positively associated with risk of CVD,[43–46] possibly as a result of serum amyloid A binding and inhibition of HDL bioactivity,[47,48] promoting monocyte chemotaxis and adhesion, and fostering proinflammatory

cytokine production,[49,50] all actions that would facilitate progression of atherosclerosis. Other experimental data demonstrate that higher cholesterol intake results in adipose tissue macrophage accumulation, which subsequently contributed to chronic inflammation,[43] and that dietary cholesterol withdrawal leads to a reduced inflammatory response and monocyte infiltration of coronary artery plaque with subsequent favorable stabilization.[51]

Extensive investigation has shown that serum cholesterol level does not directly reflect dietary cholesterol intake in healthy adults, partly owing to metabolic cholesterol homeostasis. Dietary cholesterol is absorbed in the small bowel, where it enters the portal circulation as chylomicrons, which are taken up by the liver, where cholesterol is metabolized and used for steroid biosynthesis and other biochemical requirements. The liver controls both endogenously synthesized and exogenous cholesterol and determines the amount of cholesterol released into the bloodstream in lipoproteins.[5,39,52,53] In line with substantial previous research,[54–57] our findings show that individuals with higher concentrations of serum total cholesterol experience significantly increased risk of CVD-related mortality. We found that the serum cholesterol–CVD association is largely independent of several other risk factors, including cholesterol intake. As a leading cause of death worldwide, atherosclerotic disease can be initiated by the aggregation of lipids (including circulating cholesterol) in the arterial wall, which subsequently leads to local chronic inflammation and promotes atherosclerotic plaque progression.[58] In addition to the initial plaque build-up, cholesterol can increase the production of oxysterol and aggregation, which activates arterial macrophages and an inflammatory response.[59] Circulating cholesterol can also be engulfed by arterial macrophages, promoting inflammasome activation and leading to further proinflammatory cytokine production and amplification.[3,59–61] By contrast, our results showed that men with higher serum total cholesterol level experienced lower risk of cancer mortality, and these findings are essentially in good agreement with previous studies.[62,63]

Important strengths of our study include its prospective design, large sample size, and completeness of follow-up for ascertainment of cause-specific mortality through linkage with national registries over a 31-year period. The sample size afforded considerable statistical power for the examined associations across a wide range of cohort subgroups. Our analyses included both exogenous dietary cholesterol and endogenous circulating cholesterol to offer an objective and thorough examination of the associations between cholesterol exposures and long-term health. Several study limitations should also be noted. First, we used a food frequency questionnaire to evaluate cholesterol intake and egg consumption during the previous 12 months and a single baseline measurement of serum total cholesterol level, with the risk of subsequent changes in diet and biochemical status. However, the nondifferential misclassification from inherent measurement errors would serve to underestimate the associations that influence the observed risk estimates and bias them toward the null. On the other hand, an earlier validation study using multiple-day diet records reported correlations of 0.66 for dietary cholesterol (0.67–0.75 after corrected for attenuation) and 0.58 for egg consumption, supporting the validity and reproducibility of our instrument.[25] In addition, the correlation of serum total cholesterol level between baseline and 3 years was 0.74 (Spearman correlation coefficient, $P<10^{-10}$), reflecting its stability over time. Our study was a relatively homogenous male smoker population of European ancestry with relatively high cholesterol and egg intake, which decreases generalizability of the findings to other populations. However, we included the updated meta-analysis that provided comprehensive findings from other populations. Last, we cannot rule out the potential influence of residual confounding bias of our observed associations. However, our findings remained largely unchanged after careful adjustment for a wide range of potential confounding factors, construction and use of a propensity score to control for variation of these factors among comparison groups, estimating associations on the basis of gradual increment units for exposure variables, and conducting multiple stratified analyses.

Findings from the ATBC cohort with >3 decades of observation demonstrate that greater consumption of dietary cholesterol and eggs is associated with increased risk of overall, CVD-related, and heart disease–related mortality. Increased serum total cholesterol level was also associated with increased CVD-related and heart disease–related mortality. The observed cholesterol–mortality associations were modest and independent of several other CVD risk factors. The results from this updated meta-analysis provide compelling evidence for the association between increased egg consumption and elevated risk of CVD, especially in the United States and possibly Europe, but not in Asia. Our data regarding cholesterol intake provide additional evidence relevant to dietary guidelines.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Expanded Methods

Expanded Results

Figures S1–S5

Tables S1–S18

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