ALINEA

NUTRITION

FEBRUARY 2021

www.alineanutrition.com

TABLE OF

CONTENTS

What We Know, Think We Know, or Are Starting to Know 03

Geek Box: Total Energy Intake in Nutritional Epidemiology 04

The Study 05

Results 06

The Critical Breakdown 07

Key Characteristic 07

Interesting Finding 08

Relevance 08

Application to Practice 10

References 11

Zhuang P, Wu F, Mao L, Zhu F, Zhang Y, Chen X, Jiao J, Zhang Y. Egg and cholesterol consumption and mortality from cardiovascular and different causes in the United States: A population-based cohort study. PLoS Med. 2021 Feb 9;18(2):e1003508.

What We Know, Think We Know, or Are Starting to Know

We could arguably say that of all animal-source foods, even more than red meat, eggs attract a certain level of frenzy in the diet debates. Not only does this perhaps reflect the more current debate over the role of of animal produce in the diet - see the "eggs as bad as cigarettes" narratives abounding in the plant-based world - but also the longer historic evidence linking diet and heart health.

The longer historic context is more informative for where we currently find ourselves with dietary cholesterol, which is to say at some sort of impasse. The earliest dietary public health advice for heart disease risk was to "reduce saturated fat and cholesterol", which was based primarily on the fact that these two nutrients were highly correlated: high saturated fat foods are often high cholesterol foods. It was known from tightly controlled human studies throughout the 1950' s that dietary cholesterol in fact had minimal impact on blood cholesterol levels [1-3]. Based on these studies - and more conducted over the subsequent 60yrs - it is possible to predict changes in blood cholesterol from changes in dietary cholesterol, and a reduction in dietary cholesterol of 200mg [340mg/d to 140mg/d] would reduce blood LDL-cholesterol by a mere 0.11mmol/L [4.24mg/dL]. For context, the current European Atherosclerosis Society 2019 guidelines for treating dyslipidaemia recommend achieving an LDL-C level of <2.6mmol/L (100mg/dL) , which may require a reduction of over 1.8mmol/L (70mg/dL) in some individuals [4].

There has also been a methodological challenge for cohort studies, in that dietary cholesterol intakes tend to be within fairly narrow ranges in the population, and this has often made associations difficult to detect [5]. In nutritional epidemiology, it is also important to factor in total energy intake*, and many positive findings epidemiological findings relating dietary cholesterol to disease risk did not adjust for total energy intake [6]. For eggs as a specific food, the majority of cohort studies have not found positive relationships between egg consumption and disease risk [6], and - dietary cholesterol aside - eggs are not a rich source of any particular nutrients of concern, e.g. saturated fat, sugar, trans fat, etc. However, a 2019 pooled analysis of 6 US cohorts which found a 6% increased risk for CVD per an additional half an egg, which also was no longer significant after adjusting for dietary cholesterol [7]. In this study, each additional 300mg/d dietary cholesterol was associated with a 17% increase in CVD risk.

The present study investigated the effects of eggs and dietary cholesterol in a large US cohort.

*Geek Box: Total Energy Intake in Nutritional Epidemiology

Total energy intake is a critical factor to account for in any epidemiological analysis of diet and disease for three main reasons [(8)]. First, the level of energy intake itself may be the primary factor influencing disease risk. Secondly, individuals vary in their body size and physical activity levels, and consequently in their total energy intake; this means absolute levels of nutrient intake will vary from person to person, which could introduce random error into the analysis. Finally, if a nutrient is a more direct cause of disease than total energy intake, then the effect of a nutrient of interest would be distorted by total energy intake [because as energy increases or decreases, the intake of the nutrient would increase or decrease]. The best analogy for adjusting for total energy in epidemiology is that it is seeking to achieve the same effect as having isocaloric diets in an intervention. Let's say you want to compare two diets, one high in fat and lower in carbohydrate vs. the opposite: if one diet had less energy, we would say the effects were likely due to this difference in total energy intake. This is the same for epidemiology: the exposure of interest is the composition of diet, independent of total energy intake. For epidemiology, other methods have been proposed to more assess total energy intake, in particular adjusting for body weight and adjusting for physical activity. However, these methods do not cancel out measurement errors because of body weight and physical activity are independently related to energy intake. However, because both total energy and individual nutrients are calculated from the same foods, the errors for both are strongly correlated. Therefore, by carefully adjusting the intake of a nutrient for total energy intake, these correlated errors cancel each other out and the validity of the measure of a nutrient is improved [(9)]. There are a number of methods of adjusting for total energy, including the nutrient-density method, the energy-adjusted residual method, the energy partition method, and multivariate methods. Each has certain advantages and limitations, and it is important to consider what the variable of interest is, how that variable relates to other factors, and the implications for biological plausibility of the chosen method.

The Study

The National Institutes of Health American Association of Retired Persons)Diet and Health [NIH-AARP] prospective cohort study began recruitment in 1995-1996, men and women aged between 50-71yo. Participants were recruited from 6 different states [California, Louisiana, Florida, North Carolina, New Jersey, Pennsylvania] and 2 major metropolitan areas [Atlanta, Georgia; Detroit, Michigan].

Dietary assessment used the National Cancer Institute Diet History Questionnaire, a food-frequency questionnaire [FFQ] with 124 food items, which also included questions specific to regional and ethnic considerations. The initial FFQ was validated against a subgroup of 1,953 participants who had completed the baseline FFQ, and two non-consecutive 24-hour dietary recalls that were separated by a median of 21-days.

The correlation coefficients were higher in the NIH-AARP for major nutrients than most prospective cohort studies. The correlation coefficients for eggs were 0.7 and 0.6 for men and women, respectively, and 0.7 for dietary cholesterol in both.

The primary exposures of interest in the study were intakes of whole eggs, dietary cholesterol, and egg whites/substitutes. These were calculated using the nutrient-density method, which takes absolute levels of intake and divides them by total calorie intake, and expresses the nutrients as levels per 1,000kcal. In the present study, intakes of whole eggs and dietary cholesterol were expressed as grams or milligrams per 2,000kcal. Egg whites, due to limited data, where divided dichotomously into consumers vs. non-consumers.

The primary outcomes of interest were all-cause mortality, cardiovascular disease [CVD] mortality, and cancer mortality. Analyses were adjusted for a range of dietary factors [i.e., red meat, dairy, vegetables and fruit, etc.] and non-dietary lifestyle factors [i.e., BMI, smoking, physical activity, etc.].

A number of secondary disease outcomes were also evaluated, i.e., type-2 diabetes, Alzheimer' s disease, respiratory disease, etc. For the purposes of keeping this review to a manageable length, we will confine the analysis to the primary endpoints.

Results: The analysis included 521,120 participants. Participants with higher egg consumption had higher BMI, lower income, lower education level, lower physical activity levels, higher prevalence of smoking and higher prevalence of elevated blood cholesterol levels.

The median nutrient-density intake for eggs was 8g/2,000kcal and for dietary cholesterol was 208mg/2,000kcal. Divided into quintiles, the lowest and highest quintiles of whole egg intake were 0.0g/2,000kcal and 28.7g/2,000kcal, respectively. For dietary cholesterol the lowest and highest quintiles were 118.3mg/2,000kcal and 330.0mg/2,000kcal, respectively.

The following are the results for each exposure, within each primary disease endpoint:

All Cause Mortality:

- **Whole eggs:** 28g/2,000kcal per day was associated with a 15% increased risk [HR 1.15, 95% CI 1.12-1.17]. This was no longer significant after adjusting for dietary cholesterol. Each additional half egg per day was associated with 7% [HR 1.07, 95% CI 1.06–1.08] increased risk.
- **Dietary Cholesterol:** 330mg/2,000kcal per day was associated with a 14% increased risk [HR 1.14, 95% CI 1.11-1.17]. Each additional 300mg/day dietary cholesterol was associated with a 19% [HR 1.19, 95% CI 1.16-1.22] increase in risk.
- **Egg whites/substitutes:** Compared to non-consumers, consumers had a 7% [HR 0.93, 95% CI 0.91–0.95] lower risk.

CVD Mortality:

- **Whole eggs:** Whole eggs: 28g/2,000kcal per day was associated with a 14% increased risk [HR 1.14, 95% CI 1.11-1.18]. This was no longer significant after adjusting for dietary cholesterol. Each additional half egg per day was associated with 7% [HR 1.07, 95% CI 1.06–1.09] increased risk.
- **Dietary Cholesterol:** 330mg/2,000kcal per day was associated with a 12% increased risk [HR 1.12, 95% CI 1.08-1.18]. Each additional 300mg/day dietary cholesterol was associated with a 16% [HR 1.16, 95% CI 1.11-1.21] increase in risk.
- **Egg whites/substitutes:** There was no significant association observed.

Cancer Mortality:

- **Whole eggs:** 28g/2,000kcal per day was associated with a 17% increased risk [HR 1.17, 95% CI 1.13-1.21]. This was no longer significant after adjusting for dietary cholesterol. Each additional half egg per day was associated with 7% [HR 1.07, 95% CI 1.06–1.09] increased risk.
- **Dietary Cholesterol:** 330mg/2,000kcal per day was associated with a 19% increased risk [HR 1.19, 95% CI 1.14-1.24]. Each additional 300mg/day dietary cholesterol was associated with a 24% [HR 1.24, 95% CI 1.19-1.29] increase in risk.
- **Egg whites/substitutes:** Compared to non-consumers, consumers had a 6% [HR 0.94, 95% CI 0.90–0.98] lower risk.

The Critical Breakdown

Pros: The cohort had enormous size at over half-a-million participants, long follow-up period of 16yrs, and a large number of deaths [129,328] occurred during the follow-up period from all causes, which lent substantial statistical power to the study. The statistical analysis used up to 6 models adjusting for various dietary and non-dietary lifestyle factors to determine the effects of potential confounders or effect modifiers.

Cons: The main potential issue which permeates the entire study is the use of the nutrient-density method [more under ***Key Characteristic,*** below]. For such a robust study in size, population, and calibration of the dietary assessment, it is a shame that the gold-standard validation of a 7-day measured food record was not undertaken to calibrate the initial FFQ. While the errors in a 24hr recall are independent of the errors in an FFQ, the caveat with 24hr recalls as the calibration instrument is they may result in biased estimates of the FFQ performance. There was low variability in dietary cholesterol intake, which could render more accurate associations difficult to detect.

Key Characteristic

The authors state that intakes of eggs and cholesterol were evaluated using the nutrient-density method. This is critical, and the entire study arguably turns on the use of this method. In nutritional epidemiology, it is critical to adjust for total energy intake. There are a number of reasons for this [see **Geek Box**, above], but the most important is that the effect of any nutrient could be confounded by total energy intake, i.e., total energy may be the major factor associated with increased risk for disease. Thus, in order to determine the effect of a nutrient per se, adjusting for total energy is analogous to matching diets for energy in an intervention study [8,9]. The nutrient-density method is one where the absolute intake of a nutrient is calculated, and divided by total energy intake. This is usually expressed as grams or miligrams per day, per 1,000kcal [or 2,000kcal as in the present study].

While there is simplicity in this approach, there are a number of factors which can open the nutrient-density method up to unwanted variation: where a specific nutrient has a low variability and when total energy intake is itself associated with disease [8]. Both factors are true for the present study. First, dietary cholesterol has a low variability of 118-330mg/2,000kcal. Secondly, total energy intake is itself associated with the respective disease outcomes, and dietary cholesterol highly correlated with total energy intake. In these circumstances, the nutrient-density method may often result in associations with disease outcomes even where the nutrient has no effect on the disease independent of energy intake [8].

Now, the astute will point out that total energy intake was ultimately adjusted for in the analyses [i.e., nutrient-density calculations *then adjusted additionally for total energy*]. This is known as the 'multivariate nutrient-density model', and is a useful model when there is substantial variance in energy intake. But in the present study, there is a fairly narrow contrast in energy intake and dietary cholesterol intakes. And this may not correct the fact that the primary input into the model is a nutrient-density calculated nutrient. This encapsulates the reservations with the present study, the findings of which may reflect a methodological bias.

Interesting Finding

The interaction between eggs and dietary cholesterol in this study. Recall that in the model analysing eggs and mortality outcomes, there was no significant association after adjustment for dietary cholesterol. Then, in the mediation analysis, 62.3% and 49.6% of the increase in CVD and cancer mortality, respectively, associated with whole egg intake was attributable to dietary cholesterol intake. Finally, the substitution analysis suggested that replacing half a whole egg [25g] with egg whites was associated with lower mortality risk. Putting this altogether for the association between whole eggs and mortality we are left to infer that:

- It is not other foods that correlate with eggs, like red meat, that may explain the associations
- It is not egg whites that may explain the associations

Therefore, all fingers point to the dietary cholesterol content of whole eggs being the factor associated with risk. But this is simply difficult to reconcile with the evidence from tightly controlled metabolic ward studies, from which we can accurately predict the change in blood cholesterol levels from dietary cholesterol. The addition of a half whole egg per day would be expected to increase LDL-cholesterol by 0.06mmol/L [2.4mg/dL] (4). It is difficult to attribute the increase in CVD and cancer mortality risk to such a difference. Which invites us to think about other potential mechanisms through which the dietary cholesterol content of eggs could increase risk for these diseases independent of other factors, and this is where plausible explanations become sparse.

Relevance

The major question that ensues from this study is that, in the management of chronic disease risk, are eggs particularly deleterious and does dietary cholesterol exponentially increase risk?

Although the majority of prospective cohort studies have not found any independent association between eggs and mortality, recent studies in the US have found significant, albeit weak, associations [(6)]. The fact that the HR for a half egg per day and associated confidence intervals were all within a 10% increase - and near identical for each mortality outcome - implies that relatively small effect sizes from confounders could explain away the result. Indeed, after adjusting for dietary cholesterol the relationship between eggs and the mortality outcomes was no longer significant; this is consistent with a pooled analysis of US cohorts which found a 6% increased risk for CVD per an additional half an egg, which also was no longer significant after adjusting for dietary cholesterol [(6)]. These studies cumulatively point in the direction of dietary cholesterol as the factor increasing disease risk.

The dietary cholesterol finding is perhaps more controversial, given it was the strongest association in the study. The limitation of low variability in dietary exposures (i.e., homogenous diets) was one of the rationale for the design of the NIH-AARP cohort, which sought to include participants with wide contrasts in dietary intake of foods that may be linked to cancer [(10)]. However, the main exposures for which these wide contrasts were sought were percentage of fat calories, fibre intake, fruit and vegetable intake, and red meat [(10)].

If they were to have included dietary cholesterol in the initial design rationale, it is possible that extremes of intake would have been detected in the population: the 90% percentile consume ~600mg/d while the 10th percentile consume ~75mg/d [6]. However, the majority of the population fall into a narrow range intake [6]. To quote a 1965 review of metabolic ward studies [11]: *"differences in cholesterol intake in the range of natural diets in the U.S.A. produce such small differences in the serum [cholesterol] that they are easily masked by the effects of other dietary differences which are difficult to detect or control."*

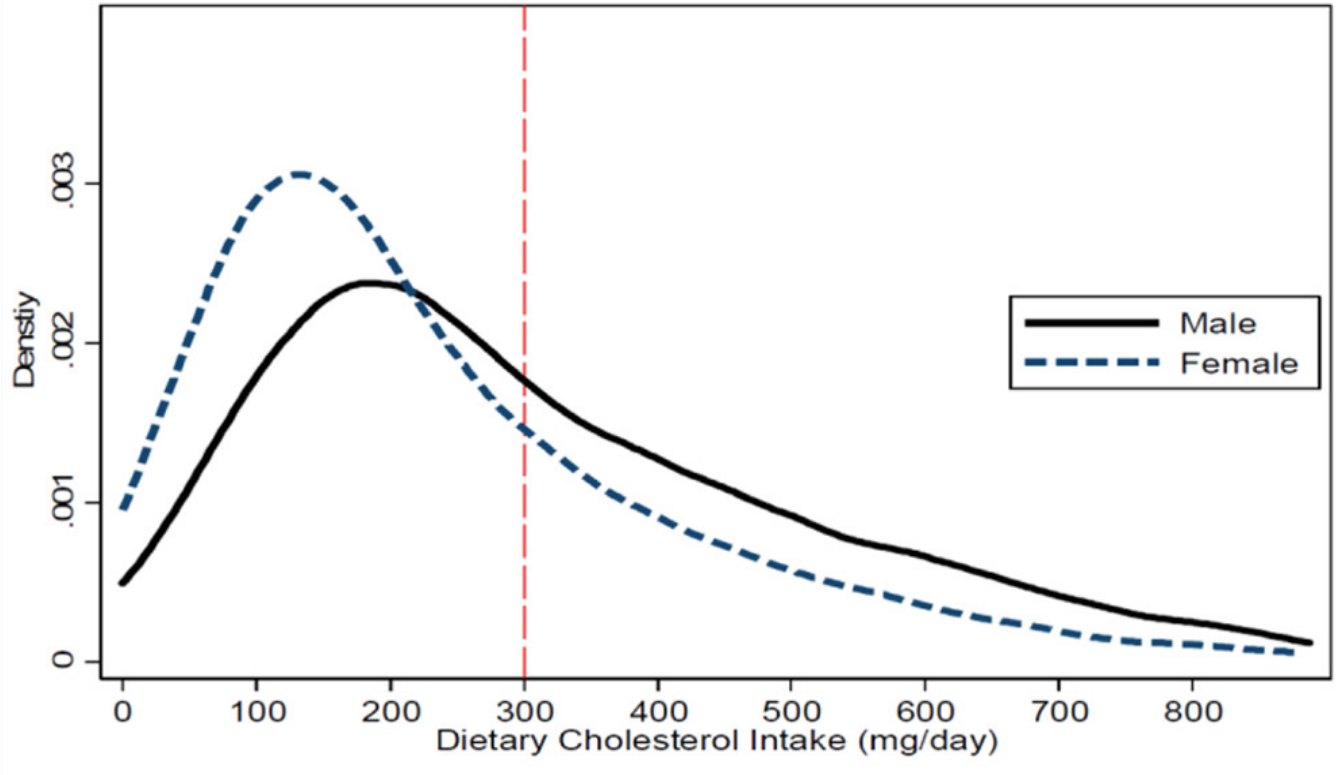

***Figure** from [6] illustrating the full range of intake of dietary cholesterol in the US population based off the most recent National Health and Nutrition Examination Survey [NHANES] data. The mean absolute intake of cholesterol is 293mg/d, which is a nutrient-density average of 137mg/1,000kcal. The curve is positively skewered, with 61% of the population within the 100-300mg/d range. Eggs contribute ~25% to dietary cholesterol intake, with the majority from meat and meat products.*

The findings of this study suggest that, within the range of dietary cholesterol intakes in the study there is a linear increase in risk, and that between 252mg/2,000kcal to 330mg/2,000kcal, risk exactly doubles for each of the all-cause, CVD, and cancer mortality outcomes, i.e., the magnitude of effect of this narrow difference in dietary cholesterol intake has identical effects on different diseases. Epidemiological findings always need to be married with biological plausibility, a mechanism identified in other lines of research that could explain an observed effect, and it is difficult to find such plausibility with this finding. For example, the difference in the amount of cholesterol actually absorbed between these 252-330mg would be 47mg of dietary cholesterol. Not only would this change blood cholesterol by a mere 0.02mmol/L [1.0mg/dL], thus rendering a doubling of CVD mortality risk beyond the bounds of plausibility, it is similarly difficult to finding a supporting case for these levels of dietary cholesterol doubling cancer mortality risk.

The majority of studies over the years which have found an association between dietary cholesterol and mortality did not adjust for total energy intake [6]. The present study did, but using the nutrient-density method as the primary input may have introduced a potential artefact in the analysis positively associated with disease risk. What this study does do is bring dietary cholesterol back into sharp focus, which warrants a reappraisal of what we think we know - and what we may not yet know - about the role of this nutrient in disease.

Application to Practice

The greatest magnitude of effect, given current levels of dietary cholesterol intake, would be to go from current averages to a zero-cholesterol diet, which would lower blood cholesterol levels by around 0.6mmol/L [24mg/dL]. Is this necessary? The weight of evidence does not suggest it is. For egg intake, it is also important to note that most positive associations are in US cohorts. Current egg intake in the UK population, for example, is no higher than an average of 2 per week. For now, factoring in the totality of evidence and not any isolated study, keep the powder dry on eliminating a nutritious food, and focus more on limiting foods rich in both saturated fat and cholesterol [like fatty red meat].

References

1. Kinsell L. Effects of High-Fat Diets on Serum Lipids; Animal vs. Vegetable Fats. Journal of the American Dietetic Association. 1954;30(7):685-8.
2. Ahrens E, Blankenhorn D, Tsaltas T. Effect on Human Serum Lipids of Substituting Plant for Animal Fat in Diet. Experimental Biology and Medicine. 1954;86(4):872-878.
3. Keys A, Anderson J, Grande F. Prediction of Serum-Cholesterol Responses of Man to Changes in Fats in the Diet. The Lancet. 1957;270(7003):959-966.
4. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ. 1997 Jan 11;314(7074):112-7.
5. Jacobs DR Jr, Anderson JT, Blackburn H. Diet and serum cholesterol: do zero correlations negate the relationship? Am J Epidemiol. 1979 Jul;110(1):77-87.
6. Carson JAS, Lichtenstein AH, Anderson CAM, Appel LJ, Kris-Etherton PM, Meyer KA, Petersen K, Polonsky T, Van Horn L, et al. Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. Circulation. 2020 Jan 21;141(3):e39-e53.
7. Zhong VW, Van Horn L, Cornelis MC, Wilkins JT, Ning H, Carnethon MR, Greenland P, Mentz RJ, Tucker KL, Zhao L, Norwood AF, Lloyd-Jones DM, Allen NB. Associations of Dietary Cholesterol or Egg Consumption With Incident Cardiovascular Disease and Mortality. JAMA. 2019 Mar 19;321(11):1081-1095.
8. Willett W. Nutritional epidemiology. New York [etc.]: Oxford University Press; 2013.
9. Rhee JJ, Cho E, Willett WC. Energy adjustment of nutrient intakes is preferable to adjustment using body weight and physical activity in epidemiological analyses. Public Health Nutr. 2014 May;17(5):1054-60.
10. Schatzkin A, Subar AF, Thompson FE, Harlan LC, Tangrea J, Hollenbeck AR, Hurwitz PE, Coyle L, Schussler N, Michaud DS, Freedman LS, Brown CC, Midthune D, Kipnis V. Design and serendipity in establishing a large cohort with wide dietary intake distributions : the National Institutes of Health-American Association of Retired Persons Diet and Health Study. Am J Epidemiol. 2001 Dec 15;154(12):1119-25.
11. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. Metabolism. 1965 Jul;14(7):759-65.