Title:
Egg intake and cancers of the breast, ovary, and prostate: dose-response meta-analysis of prospective observational studies

N. Keum,[1,2] D.H. Lee,[1,2] N. Marchand,[1] H. Oh,[1,2] H. Liu,[3] D. Aune,[4,5] D.C. Greenwood,[6] E.L. Giovannucci[1,2,7]

1. Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, USA
2. Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, USA
3. Department of Epidemiology and Health Statistics, Zhejiang University, Zhejiang, China
4. Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway
5. Department of Epidemiology and Biostatistics, Imperial College London, London, UK
6. Division of Biostatistics, University of Leeds, Leeds, UK
7. Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA

Corresponding author:
ScD NaNa Keum
Departments of Nutrition and Epidemiology
Harvard School of Public Health
Building 2, 3rd Floor
655 Huntington Avenue, Boston, MA, 02115, USA
Phone: +1-617-432-4648
Fax: +1-617-432-2435
E-mail: nak212@mail.harvard.edu

Running title:
Egg intake and sex-hormone related cancers

Abstract

Evidence suggests that egg intake may be implicated in the aetiology of sex-hormone related cancers. However, dose-response relationships between egg intake and such cancers are unclear. Thus, we conducted dose-response meta-analyses to summarize the dose-response relationships between egg consumption and risks of breast, prostate, and gynecological cancers. PubMed and Embase were searched up to April, 2015 to identify relevant prospective observational studies. Summary relative risk (RR) and 95% confidence interval (CI) were estimated using a random-effects model. For breast cancer, linear dose-response meta-analysis found a non-significantly increased risk (RR for an increase of 5 eggs/week: 1.05, 95% CI 0.99, 1.11, 16,023 cases). Albeit evidence for non-linearity was not statistically significant ($P_{non-linearity}$=0.50, 15,415 cases), consuming $\geq$ 5 eggs per week was associated with a statistically significantly increased risk compared to no egg consumption, with RR being 1.04 (95% CI 1.01, 1.07) at 5 eggs/week and 1.09 (95% CI 1.03, 1.15) at about 9 eggs/week. For other cancers investigated, the summary RR for an increase of five eggs consumed per week was 1.08 (95% CI 1.00, 1.17, 2,924 cases) for ovarian cancer and 1.47 (95% CI 1.01, 2.14, 609 cases) for fatal prostate cancer with evidence of small study effects (P_{Egger}=0.04). No evidence of an association was found for total prostate cancer. While potential for publication bias and confounding temper our conclusion, high egg intake may be associated with a modestly elevated risk of breast cancer; a positive association of egg intake with ovarian and fatal prostate cancers cannot be ruled out.

Introduction

Eggs are frequently consumed worldwide, with an estimated number of eggs consumed per person over a year ranging from a low of 47 in India to a high of 349 in China in 2007 (of note, US=250;UK=178).[1] Eggs are low in saturated fat and have a high nutritional value supplying high-quality protein, mono- and poly-unsaturated fats, vitamins (A, B, and D) and minerals (e.g. iron).[2] Yet, their high contents of cholesterol (186 mg per a 50g egg[2] vs. 300 mg Reference Daily Intake[3]) and choline (126 mg per a 50g egg[2] vs. 425-550 mg/day Adequate Intake for women and men aged ≥19 years[4]) lend biological plausibility to a possible role of egg consumption in the aetiology of sex-hormone related cancers. Cholesterol serves as a precursor for sex hormones such as androgens and estrogens[5] that promote cell proliferation, thereby contributing to carcinogenesis of breast, prostate, and gynecologic cancers.[6; 7; 8] Evidence also suggests that 27-hydroxycholesterol, a primary metabolite of cholesterol, plays a role as estrogen receptor agonist in breast cancer cells, mimicking the effects of estrogen.[9; 10] Choline has been particularly implicated in prostate cancer proliferation and progression through its role in cell membrane synthesis and function,[11] although choline, as a methyl donor, has been suggested to be inversed associated with breast cancer risk.[12] Furthermore, given that a 50g egg contains only 78 calories, [2] eggs provide a considerable amount of protein per calorie (6 g per 78 calories[2] vs. 50g Reference Daily Intake[3]). High protein intake, particularly high-quality proteins,[13; 14] increases the production of insulin-like growth factor 1 (IGF-1) that promotes tissue growth and tumour progression.[13; 15; 16] Lastly, chlorine used to wash eggs before commercial sale may enter eggs through the pores in shells and interact with eggs' organic substances, converting to potentially carcinogenic organochlorines. Organochlorines disrupt estrogen-related pathways, thereby implicated in carcinogenesis of gynecological cancers, particularly breast cancer.[17]

Indeed, several meta-analyses of observational studies have assessed a potential harm of egg intake on such cancers and found a positive association with breast[18] and ovarian[19] cancer risks, some suggestion of an increased risk for endometrial[20] and fatal prostate cancers,[21] but no association with total prostate cancer risk.[21] However, given that distribution of egg intake differs across studies, previous meta-analyses[19; 20; 21] that pooled RRs comparing the highest vs. lowest category of egg intake have limited interpretability in terms of dose-response relationship.

To better guide dietary recommendation for egg intake, it remains to be answered if cancer risk increases with each additional egg intake or if there is a range of egg intake that does not elevate cancer risk. While meta-analysis on breast cancer[18] addressed the aforementioned problem by pooling RRs separately for distinctive range of egg intake (e.g. 1-<2 vs. <1, 2-5 vs. <1, >5 vs. <1 egg/week), it included case-control studies with a high potential for recall and selection bias to distort the true diet-cancer relationship. While the World Cancer Research Fund /American Institute for Cancer Research (WRCF/AICR) Expert Panel assessed the dose-response relationships between egg intake and cancers of the ovary[22] and prostate[23] based on prospective studies published up to December 2012 and April 2013, respectively, they concluded evidence for an association to be 'limited-no conclusion.' More studies may have been published since their search and updated analyses will help better evaluate the evidence. Thus, based on prospective observational studies, we conducted dose-response meta-analysis to quantify the amount of egg intake associated with an increase in the risk of breast, prostate, and gynecologic cancers and to identify the shape of the relationships.

Methods

For the design, analysis, and reporting of this study, standard guidelines for meta-analysis of prospective observational studies was followed. [24] Three authors (DL, HL, NM) participated in

literature search, study selection, and data extraction independently. Inconsistency between researchers was resolved through discussion.

Literature Search

PubMed and Embase databases were searched for studies through December 2014 and the search was later updated through April 2015. Detailed search terms are provided (Supplementary Table 1). The language was limited to English and no other restrictions were imposed. Abstracts and unpublished results were not included. The reference lists of selected reviews and meta-analyses, and all the articles included in our analysis were also reviewed for additional studies.

Study Selection

To be included, studies had to be a prospective observational study (i.e. a cohort study analysed with nested case-control, case-cohort, or prospective cohort approaches) or a pooled study analysed prospectively on the relationship between egg intake and incidence of breast, prostate, and gynecologic cancers. A pooled study was considered for inclusion, as not all cohorts included in a pooled study published the results independently. Retrospective case-control studies were excluded to minimize the impact of recall bias and selection bias. Of note, prostate cancer is a highly heterogeneous disease and over detection of indolent prostate cancer often dilutes the exposure and outcome relationship in epidemiologic studies. Thus, we included studies on fatal prostate cancer as well in order to examine the effect of egg intake on aggressive prostate cancer separately.

For dose-response meta-analysis, studies had to provide the following information: a quantitative measure (e.g., g/day or week, number/day or week, serving/day or week) of egg intake for at least 3 categories with the estimates of relative risks (RRs) (rate ratio, or hazard ratio), 95% confidence interval (CI), category-specific or total number of cases, and category-specific or total number of either noncases or person-years. When studies did not provide the aforementioned information but directly reported RR and 95% CI for the linear effect of egg intake, they were included in the linear dose-response meta-analysis. Authors of two studies[25; 26] were contacted to obtain data on category-specific number of person-years or cases and they provided the requested information.

Data Extraction

From each study, the following information was extracted: the most fully adjusted RR and corresponding 95% CI in each category of egg intake, category-specific range of egg intake, unit of egg intake, category-specific or total number of cases, category-specific or total number of person-years or noncases, first author's name, publication year, study design, study period, characteristics of the study population (cohort name, country, sex, age at enrollment), dietary assessment method (type, whether it had been validated), adjustment variables.

Statistical Analysis

Dose-response meta-analyses consist of two parts: linear and non-linear analysis. For linear dose-response meta-analysis, assuming a linear relationship between egg intake and cancer risk, the method described by Greenland and Longnecker[27] was used to calculate study-specific RRs (linear slopes) and 95% CIs from the correlated RRs and 95% CIs extracted across categories of egg intake. In estimating study-specific linear trends, several approximations were made: the midpoint of egg

intake in each category was assigned to the corresponding RR; the width of the open-ended highest category was assumed to be the same as that of the adjacent interval; when the distributions of person-years or non-cases were not provided but analysed based on quantiles, they were equally divided across the quantiles. Egg intake reported in (serving or g) /(day or week) was converted to number of eggs/week, by assuming the weight of one egg as 50g (equivalent to one large egg) and the portion size of one serving as one egg. Then, the estimated study-specific RRs and variances were pooled using DerSimonian–Laird random effects model to calculate the summary RR and 95% CI. Forest plots of the linear dose-response meta-analysis were presented for RRs for an increase of five eggs consumed per week.

To explore potential non-linear relationship between egg intake and cancer risk, non-linear dose-response meta-analysis was performed based on the restricted cubic spline approach.[28; 29] For each study, cubic splines were modeled with three knots fixed at percentiles (10%, 50%, and 90%) of the whole distribution of egg intake contributed by all included studies, accounting for correlation across category-specific RRs and 95% CIs within each study.[28] The reference was set to 0 eggs/day, the lowest value of the reported egg intakes. Then, the derived curves were combined using multivariate DerSimonian–Laird random effects meta-analysis.[30] The p-value for nonlinearity was obtained from the test of the null hypothesis that the regression coefficient of the second spline transformation was equal to zero. Of note, the cubic spline approach requires that studies analyse egg intake in more than three categories. To allow adequate information for the robust estimation of the curve, this non-linear meta-analysis was applied when five or more cohorts contributed data to non-linear dose-response meta-analysis.

Heterogeneity in the relationship between egg intake and cancer risk across studies was assessed by Cochran's Q test[31] and quantified by the percentage of total variation across studies that is attributable to between-study heterogeneity (I^2).[32] No subgroup analyses and meta-regression were conducted due to the small number of studies included and upon observing no evidence of heterogeneity in most analyses. Potential for small study effects,[33; 34] such as publication bias, was assessed visually using funnel plot and statistically using Egger's test[35] with awareness that the statistical power is limited when the number of studies included is small. To explore robustness of the results, diverse sensitivity analyses were performed such as assuming one serving size of egg intake as two eggs for studies that did not report or did not specify the serving size of egg intake on food frequency questionnaires and assessing the influence of individual studies on the pooled estimate by excluding each study in turn. For statistical significance, two-sided α was set at 0.05. All statistical analyses were conducted using STATA 12 (StataCorp, College Station, TX).

Results

      The results of the literature search and study selection are summarized in Figure 1. Out of 7,378 publications screened, a total of 19 publications[25; 26; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52] were included in this dose-response meta-analysis. For gynecologic cancers, no studies met our inclusion criteria, except for ovarian cancer. Thus, this dose-response meta-analysis examined three cancer site (breast, ovary, prostate) in relation to egg intake.

      Characteristics of the 19 included studies are summarized in Supplementary Table 2. Six out of the 19 publications were on breast cancer, of which one was a pooled study. Among the breast cancer studies, three were from the U.S., two from Europe, and one from Asia; one study was restricted to premenopausal women while the rest included both premenopausal and postmenopausal women. Three out of the 19 publications were on ovarian cancer, of which one was a pooled study. Among the ovarian cancer studies, one was from the U.S. and two from Europe; all studies included a mixed population of premenopausal and postmenopausal women. Ten out of the 19 publications were on prostate cancer, of which six were on total prostate cancer and four on fatal prostate cancer. Among the prostate cancer studies, six were conducted in the U.S., two in Europe, and two in Asia. While all studies on breast and ovarian cancers were adjusted for multiple potential confounders, three studies on prostate cancer were adjusted for age only.

Breast Cancer

      In the linear dose-response meta-analysis, five cohort studies[36; 37; 38; 39; 40] and one pooled study[26] of five cohort studies were included, involving a total of 16,023 cases with category-specific midpoints of egg consumption ranging from 0 to 8.8 eggs/week. The summary RR for an increase of five eggs consumed per week was 1.05 (95% CI=0.99-1.11), with no evidence of heterogeneity (I^2=0%, $P_{heterogeneity}$=0.93) (Figure 2A). The results did not change materially when the serving size of egg intake was set to two eggs rather than one egg for studies[37; 38; 39] that did not report or specify the serving size of egg intake on food frequency questionnaires (data not shown). In sensitivity analyses omitting one study at a time, the results were robust to the influence of any single study included. Small study effects, such as publication bias, were not indicated (P_{Egger}=0.62)

      The non-linear dose-response meta-analysis was conducted after excluding two studies[38; 39] that analysed egg intake in three categories only (15,415 cases, range=0-8.8 eggs/week). While there was no evidence of non-linearity ($P_{non-linearity}$=0.50) (Figure 2B), egg intakes of $\geq$ 5 eggs/week was associated with statistically-significant, increased risk of breast cancer. Approximately, compared to 0 egg/week, the summary RR was 1.04 (95% CI=1.01-1.07) at 5 eggs/week and further increased to 1.09 (95% CI=1.03-1.15) at about 9 eggs/week. In sensitivity analyses such as assuming the serving size of egg consumption as two eggs for the study by Gaard et al.[37] and excluding the study,[37] the results were consistent with the risk increasing statistically significantly by 4% starting from 5 eggs/week consumed (data not shown).

Ovarian Cancer

      In the linear dose-response meta-analysis, a pooled study[41] of 11 cohorts and two independent cohort studies[42; 43] (2,924 cases, range of median egg intake of cohorts in the pooled analysis=0.8-3 eggs/week, category-specific midpoints of egg intake in two independent studies=0.5-5 eggs/week) were included. An increase of five eggs consumed per week was associated with an approximately 8% increased risk of ovarian cancer with borderline statistical significance (RR=1.08, 95% CI=1.00-1.17)

with no evidence of heterogeneity (I^2=0%, $P_{heterogeneity}$=0.59) (Figure 3A). As the pooled study had a dominant influence on the summary estimate, assuming the serving size of egg consumption as two eggs for the study by Larsson[42] did not change the results. Small study effects, such as publication bias, were not indicated (P_{Egger}=0.60).

The non-linear dose-response meta-analysis could not be performed, because the pooled study[41] did not report results for more than three categories of egg intake, leaving only two cohort studies eligible for the cubic spline approach.

Prostate Cancer

A total of 10 studies[25; 44; 45; 46; 47; 48; 49; 50; 51; 52] were eligible for dose-response meta-analysis, of which six studies[44; 45; 48; 49; 50; 52] investigated total prostate cancer (3,655 cases, range=0.5-7 eggs/day) and the remaining four studies[25; 46; 47; 51] examined fatal prostate cancer (609 cases, range=0.3-6.8 eggs/day).

For total prostate cancer, there was no evidence of a linear association (RR=1.00, 95% CI=0.88-1.14, I^2=0%, $P_{heterogeneity}$=0.69) (Figure 4). In sensitivity analyses such as assigning two eggs to each frequency of egg consumption for studies[45; 48; 50] that needed the serving size assumption and omitting one study at a time, the results did not change materially. Small study effects, such as publication bias, were not indicated (P_{Egger}=0.72).

In contrast, for fatal prostate cancer, an increase of five eggs consumed per week was associated with an approximately 47% elevated risk (RR=1.47, 95% CI=1.01-2.14, I^2=40%, $P_{heterogeneity}$=0.17) (Figure 4). When the serving size of egg consumptions was changed from one egg to two eggs for studies[46; 47; 51] that needed such assumption, the effect size was attenuated (RR=1.23, 95% CI=0.97-1.55, I^2=70%, $P_{heterogeneity}$=0.08). In sensitivity analyses omitting one study at a time, a direct linear association persisted across each exclusion but statistical significance was lost in all but exclusion of the study by Hsing et al.[46] Evidence of small study effects, such as publication bias, was indicated (P_{Egger}=0.04) with relatively smaller studies reporting a stronger linear association (Figure 5).

Non-linear dose-response relationship was not explored due to insufficient number of studies eligible for the robust estimation of the curve, which was specified a priori in the method section.

Discussion

Frequently consumed worldwide, eggs have high contents of cholesterol and choline and provide relatively high protein per calories, all of which may link egg consumption to risks of breast, ovary, and prostate cancers. Yet, most of the previous studies on such cancers have not investigated egg consumption as the primary exposure of interest, limiting a rigorous evaluation of the hypothesized associations. In our linear dose-response meta-analyses of prospective observational studies, we found a statistically non-significant positive association with breast cancer and a possible positive association with ovarian and fatal prostate cancers, with an increase of five eggs consumed per week elevating the risk by 5%, 8% and 47%, respectively. No evidence of a linear association was found with total prostate cancer. For breast cancer for which non-linear dose-response meta-analysis was performed, whilst evidence of non-linearity was not statistically significant, the curve showed an upward tendency, with women consuming five or more eggs per week having a statistically-significant but modestly elevated risk compared with non-consumers.

To date, one meta-analysis[18] and one pooled study[26] have been conducted to assess the relationship between egg intake and breast cancer. While findings from the two different methods of analysis are conflicting, our study may provide some clues to reconcile such inconsistency. The pooled analysis[26] of five cohort studies suggested a J-shape association with the risk decreasing for <0-<2 eggs/week (RR=0.93, 95% CI=0.82-1.05) but increasing for >7 eggs/week (RR=1.07, 95% CI=0.90-1.28) compared to 0 egg/week. However, the past meta-analysis[18] that included the pooled study, cohort studies, and case-control studies did not confirm the J-shape association and reported an increased risk only in categories of 2-5 eggs/week relative to <1 egg/week. Albeit evidence for non-linearity was not statistically significant, our study based on the pooled study and cohort studies found some upward tendency to the dose-response curve, with the risk elevating statistically-significantly for ≥5 eggs/week relative to 0 egg/week, which is more consistent with the results from the pooled analysis. Based on more than twice as many cases as the pooling project and with more data over the range of 3-6 eggs/week contributed by the additional cohort studies, our analysis had richer information to examine the dose-response relationship. Inconsistency with the previous meta-analysis may be partially explained by its inclusion of case-control studies that are more prone to recall and selection biases and by its failure to account for correlation across RRs within the same study (e.g. two RRs from the same study were pooled together with RRs from other studies using a random effect model).

For ovarian cancer, while no new study was identified to update the previous dose-response meta-analysis by the WRCF/AICR,[53] our analysis based on different inclusion criteria concerning the pooled analysis[41] may contribute to raising the level of evidence for a positive association from the current "limited-no conclusion" to "limited-suggestive." Three[54; 55; 56] out of the 11 cohorts included in the pooled analysis published the results separately. Unlike our analysis that included the pooled study[41] itself, the analysis by the WRCF/AICR used these three studies.[54; 55; 56] As eggs are a source of protein and fat, the relationship between egg intake and ovarian cancer risk has been investigated primarily in the context of examining the effect of either protein or fat intake on ovarian cancer risk. Thus, studies with a null finding would have been less likely to be published, which is a major source of publication bias in meta-analysis. Indeed, two[54; 55] of the three studies[54; 55; 56] published independently from the pooled study[41] reported a statistically significant, strong association. Additionally, as a pooled study combines data from the participating cohorts in a standardized manner, inclusion of pooled studies in meta-analyses help reduce artificial heterogeneity arising from methodological differences across studies. The RR reported by the WRCF/AICR, after converted to RR for an increase of 5 eggs consumed per week, was 1.30 (95% CI=0.93-1.82, I^2=46%), which is stronger in the strength of association but less precise and more heterogeneous compared to our findings (RR=1.08, 95% CI=1.00-1.17, I^2=0%).

Pertaining to prostate cancer, due to lack of additional studies published, our findings are virtually identical to those reported by the WRCF/AICR,[57] suggesting that egg intake may have no effect on total prostate cancer but possibly increase the risk of fatal prostate cancer. While evidence of small study effects and lack of comprehensive adjustment for potential confounders in the studies included warrant cautious interpretation, the observed positive association between egg intake and fatal prostate cancer is biologically plausible, because cholesterol and, particularly, choline have been implicated in prostate cancer progression. In addition to serving as a precursor to androgens that promote prostate cancer growth, cholesterol is an essential component of animal cell membranes.[5] Thus, a large amount of cholesterol is required for malignant cells to support their rapid growth and proliferation.[58] In prostate cells, cholesterol accumulates as the cells transform to malignant tumours.[59] The use of a cholesterol lowering drug, statins, was statistically significantly associated with an approximately 20% reduced risk of advanced prostate cancer.[60] Similarly, choline, through its

conversion to a phospholipid by choline kinase, plays an important role in cell membrane synthesis and function, thereby implicated in cancer proliferation and progression.[11] Malignant prostate cells overexpress choline transporter and kinase for an increased choline uptake and metabolism[11; 61] and choline is more highly concentrated in malignant than in normal prostate cells.[62] Considering that choline is an essential nutrient that must be consumed through diet and that eggs are a major contributor to choline intake,[63] contribution of choline appears to be particular important in linking egg intake and fatal prostate cancer.

Our meta-analysis has several limitations. First, measurement error in egg intake is of particular concern. Given growing egg consumption globally,[1] participants may have changed their egg consumption over time. However, most of the included studies with a long-term follow-up based their analysis on baseline egg intake only. Additional measurement errors were introduced during dose-response meta-analysis due to inevitable assumptions such as assigning one egg to each serving when studies did not report or specify the serving size of egg intake on food frequency questionnaires; using the midpoint of egg intake in each category as the dose for corresponding RR; approximating the width of open-ended highest category from the adjacent interval. Such inevitable measurement error from the diverse sources could bias the results in either direction, but are generally anticipated to attenuate the true effect,[64] particularly since the dietary information was collected before participants' knowledge of case status. The concern is further alleviated given the consistency in our results regardless of whether one egg or two eggs were assigned to each serving of egg intake for some studies that needed the serving size assumption.

Second, while the proportion of eggs consumed as a component of recipes rather than in-shell is increasing, especially in developed countries,[1] most of the included studies analysed only egg consumed as such, underestimating the true egg intake. Thus, our linear dose-response may have overestimated the true association and, particularly for breast cancer for which non-linear relationship was explored, the cut-off point from which a statistically- significantly elevated risk starts may occur at total egg intake greater than ≥ 5 eggs/week. However, as egg consumed as such is likely to be the major determinant of the variation in total egg intake, quantifying cancer risk based on egg consumption as such still provides useful information.

Third, most of the studies included did not adjust for dietary factors. While not many dietary factors are established risk factors for the cancers examined, people eat a food item not in isolation but in combination with other foods. Thus, as a potential explanation for the statistically-significant associations observed between egg intake and risks of breast and fatal prostate cancers, confounding by foods (e.g. red meat) correlated with egg intake cannot be ruled out completely.

Finally, while there was no evidence of statistical heterogeneity in summary RRs for breast, ovarian, and total prostate cancers, we were not able to explore some important biological heterogeneity due to lack of data. For instance, it was reported that the association of egg intake and breast cancer risk was stronger among pre-menopausal women than postmenopausal women[65]; that egg intake was specifically associated with breast cancer with positive estrogen and progesterone receptors.[66] Furthermore, in light of the evidence that an increment in serum cholesterol for a given dietary cholesterol intake diminishes with increasing baseline dietary cholesterol intake[67] and that diabetic people are more responsive to dietary cholesterol intake,[68] if cholesterol is the main mediator of the relationship between egg intake and cancer risk, the association might be stronger in populations with otherwise low dietary cholesterol intake or in diabetic patients.

Yet, there are several strengths in our meta-analysis as well. To date, this is the first meta-analysis that attempted to summarize the dose-response relationships of egg intake with breast, prostate, and gynecologic cancers. As we excluded case-control studies, our findings are more robust against

recall bias and selection bias. Low heterogeneity observed in most of the analyses increases the generalizability of our findings. By preferentially incorporating pooled studies where possible, our estimates had more precision, less unwanted heterogeneity, and more immunity against publication bias than those that would have been obtained by incorporating selectively published studies from a few cohorts included in a pooled study. Finally, our dose-response meta-analyses for total prostate cancer included studies that were conducted in Europe and Asia where PSA screening rate is relatively low and in the US before 1994 when only a minority of men was screened for PSA. Hence, total prostate cancer is likely to represent more of clinically significant prostate cancer rather than indolent prostate cancer.

In conclusion, consuming ≥5 eggs per week may be associated with an elevated risk of breast cancer compared to no egg consumption. Our study provides only limited evidence to support a direct linear association between egg intake and the risk of ovarian and fatal prostate cancers. While potential for publication bias and confounding by other foods temper our conclusion, we cannot rule out an association. Considering that eggs are frequently consumed worldwide, more prospective studies primarily investigating the effect of egg consumption on these cancer risks are warranted to confirm these associations.

Financial support
None declared.

Disclosure
The authors declared no conflicts of interest.

Authorship
NK designed the study, analysed and interpreted the data, and drafted the manuscript. DHL acquired the data, analysed and interpreted the data. NM acquired the data, analysed and interpreted the data. HO acquired the data, analysed and interpreted the data. HL acquired the data, analysed and interpreted the data. DA interpreted the data and drafted manuscript. DCG wrote the statistical analysis code, interpreted the data, and drafted manuscript. ELG was the principal investigator; designed the study, interpreted the results, and drafted the manuscript.

References

1. Food and Agriculture Organization of the United Nations. Agribusiness Handbook: Poultry Meat and Eggs. Available at: https://www.responsibleagroinvestment.org/node/738, accessed on September 1, 2014.
2. U.S. Department of Agriculture, Agricultural Research Service, 2010. USDA National Nutrient Database for Standard Reference, Release 23, Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata.

3. USDA (January 2013) Guidance for industry: a food labeling guide (14. Appendix F: calculate the percent daily value for the appropriate nutrients).

4. Food and Nutrition Board of Institute of Medicine, National Academies. Dietary Reference Intakes for Folate and Other B Vitamins. Available at:

http://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx, accessed on September 1, 2014.

5. Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. Section 26.4, Important Derivatives of Cholesterol Include Bile Salts and Steroid Hormones. Available from: http://www.ncbi.nlm.nih.gov/books/NBK22339/.

6. Henderson BE, Feigelson HS (2000) Hormonal carcinogenesis. *Carcinogenesis* **21**, 427-433.

7. Bosland MC, Mahmoud AM (2011) Hormones and prostate carcinogenesis: Androgens and estrogens. *Journal of carcinogenesis* **10**, 33.

8. Yager JD, Davidson NE (2006) Estrogen carcinogenesis in breast cancer. *The New England journal of medicine* **354**, 270-282.

9. Nelson ER, Wardell SE, Jasper JS *et al.* (2013) 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* **342**, 1094-1098.

10. DuSell CD, Umetani M, Shaul PW *et al.* (2008) 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Molecular endocrinology* **22**, 65-77.

11. Awwad HM, Geisel J, Obeid R (2012) The role of choline in prostate cancer. *Clinical biochemistry* **45**, 1548-1553.

12. Zhang CX, Pan MX, Li B *et al.* (2013) Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. *Cancer science* **104**, 250-258.

13. Giovannucci E, Pollak M, Liu Y *et al.* (2003) Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **12**, 84-89.

14. Maskarinec G, Takata Y, Murphy SP *et al.* (2005) Insulin-like growth factor-1 and binding protein-3 in a 2-year soya intervention among premenopausal women. *The British journal of nutrition* **94**, 362-367.

15. Giovannucci E (1999) Insulin-like growth factor-I and binding protein-3 and risk of cancer. *Hormone research* **51 Suppl 3**, 34-41.

16. Underwood LE, Thissen JP, Lemozy S *et al.* (1994) Hormonal and nutritional regulation of IGF-I and its binding proteins. *Hormone research* **42**, 145-151.

17. Salehi F, Turner MC, Phillips KP *et al.* (2008) Review of the etiology of breast cancer with special attention to organochlorines as potential endocrine disruptors. *Journal of toxicology and environmental health Part B, Critical reviews* **11**, 276-300.

18. Si R, Qu K, Jiang Z *et al.* (2014) Egg consumption and breast cancer risk: a meta-analysis. *Breast Cancer* **21**, 251-261.

19. Zeng ST, Guo L, Liu SK *et al.* (2014) Egg consumption is associated with increased risk of ovarian cancer: Evidence from a meta-analysis of observational studies. *Clinical nutrition*.

20. Bandera EV, Kushi LH, Moore DF *et al.* (2007) Consumption of animal foods and endometrial cancer risk: A systematic literature review and meta-analysis. *Cancer Causes and Control* **18**, 967-988.

21. Xie B, He HD (2012) No Association between Egg Intake and Prostate Cancer Risk: A Meta-analysis. *Asian Pacific Journal of Cancer Prevention* **13**, 4677-4681.

22. World Cancer Research Fund / American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Ovarian Cancer 2014. Available at http://www.dietandcancerreport.org/cup/cup_resources.php.

23. World Cancer Research Fund International/American Institute for Cancer Research. Continuous Update Project Report: Diet, Nutrition, Physical Activity, and Prostate Cancer. 2014. Available at: www.wcrf.org/sites/default/files/Prostate-Cancer-2014-Report.pdf.

24. Stroup DF, Berlin JA, Morton SC *et al.* (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* **283**, 2008-2012.

25. Richman EL, Kenfield SA, Stampfer MJ *et al.* (2011) Egg, red meat, and poultry intake and risk of lethal prostate cancer in the prostate-specific antigen-era: incidence and survival. *Cancer prevention research* **4**, 2110-2121.

26. Missmer SA, Smith-Warner SA, Spiegelman D *et al.* (2002) Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. *International journal of epidemiology* **31**, 78-85.

27. Greenland S, Longnecker MP (1992) Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* **135**, 1301-1309.

28. Harrell FE, Jr., Lee KL, Pollock BG (1988) Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst* **80**, 1198-1202.

29. Orsini N, Greenland S (2011) A procedure to tabulate and plot results after flexible modeling of a quantitative covariate. *Stata Journal* **11**, 1-29.

30. White IR (2009) Multivariate random-effects meta-analysis. *Stata Journal* **9**, 40-56.

31. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* **21**, 1539-1558.

32. Higgins JP, Thompson SG, Deeks JJ *et al.* (2003) Measuring inconsistency in meta-analyses. *Bmj* **327**, 557-560.

33. Sterne JA, Gavaghan D, Egger M (2000) Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *Journal of clinical epidemiology* **53**, 1119-1129.

34. Nuesch E, Trelle S, Reichenbach S *et al.* (2010) Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. *BMJ* **341**, c3515.

35. Harbord RM, Egger M, Sterne JAC (2006) A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Statistics in medicine* **25**, 3443-3457.

36. Farvid MS, Cho E, Chen WY *et al.* (2014) Dietary protein sources in early adulthood and breast cancer incidence: prospective cohort study. *Bmj* **348**, g3437.

37. Gaard M, Tretli S, Loken EB (1995) Dietary fat and the risk of breast cancer: a prospective study of 25,892 Norwegian women. *International journal of cancer Journal international du cancer* **63**, 13-17.

38. Key TJ, Sharp GB, Appleby PN *et al.* (1999) Soya foods and breast cancer risk: a prospective study in Hiroshima and Nagasaki, Japan. *British journal of cancer* **81**, 1248-1256.

39. Mills PK, Beeson WL, Phillips RL *et al.* (1989) Dietary habits and breast cancer incidence among Seventh-day Adventists. *Cancer* **64**, 582-590.

40. Pala V, Krogh V, Berrino F *et al.* (2009) Meat, eggs, dairy products, and risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Am J Clin Nutr* **90**, 602-612.

41. Genkinger JM, Hunter DJ, Spiegelman D *et al.* (2006) A pooled analysis of 12 cohort studies of dietary fat, cholesterol and egg intake and ovarian cancer. *Cancer causes & control : CCC* **17**, 273-285.

42. Larsson SC, Wolk A (2005) No association of meat, fish, and egg consumption with ovarian cancer risk. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **14**, 1024-1025.

43. Schulz M, Nothlings U, Allen N *et al.* (2007) No association of consumption of animal foods with risk of ovarian cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **16**, 852-855.

44. Allen NE, Key TJ, Appleby PN *et al.* (2008) Animal foods, protein, calcium and prostate cancer risk: the European Prospective Investigation into Cancer and Nutrition. *British journal of cancer* **98**, 1574-1581.

45. Allen NE, Sauvaget C, Roddam AW *et al.* (2004) A prospective study of diet and prostate cancer in Japanese men. *Cancer causes & control : CCC* **15**, 911-920.

46. Hsing AW, McLaughlin JK, Schuman LM *et al.* (1990) Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. *Cancer research* **50**, 6836-6840.

47. Iso H, Kubota Y, Japan Collaborative Cohort Study for Evaluation of C (2007) Nutrition and disease in the Japan Collaborative Cohort Study for Evaluation of Cancer (JACC). *Asian Pacific journal of cancer prevention : APJCP* **8 Suppl**, 35-80.

48. Mills PK, Beeson WL, Phillips RL *et al.* (1989) Cohort study of diet, lifestyle, and prostate cancer in Adventist men. *Cancer* **64**, 598-604.

49. Schuurman AG, van den Brandt PA, Dorant E *et al.* (1999) Animal products, calcium and protein and prostate cancer risk in The Netherlands Cohort Study. *British journal of cancer* **80**, 1107-1113.

50. Severson RK, Nomura AM, Grove JS *et al.* (1989) A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer research* **49**, 1857-1860.

51. Snowdon DA, Phillips RL, Choi W (1984) Diet, obesity, and risk of fatal prostate cancer. *Am J Epidemiol* **120**, 244-250.

52. Thompson MM, Garland C, Barrettconnor E *et al.* (1989) Heart-Disease Risk-Factors, Diabetes, and Prostatic-Cancer in an Adult Community. *American Journal of Epidemiology* **129**, 511-517.

53. World Cancer Research Fund / American Institute for Cancer Research. WCRF/AICR Systematic Literature REview Continuous Update Project Report. The Associations between Food, Nutrition, Physical Activity, and the Risk of Ovarian Cancer 2013. Available at http://www.wcrf.org/sites/default/files/Ovarian-Cancer-SLR-2013.pdf.

54. Bertone ER, Rosner BA, Hunter DJ *et al.* (2002) Dietary fat intake and ovarian cancer in a cohort of US women. *Am J Epidemiol* **156**, 22-31.

55. Kushi LH, Mink PJ, Folsom AR *et al.* (1999) Prospective study of diet and ovarian cancer. *Am J Epidemiol* **149**, 21-31.

56. Kiani F, Knutsen S, Singh P *et al.* (2006) Dietary risk factors for ovarian cancer: the Adventist Health Study (United States). *Cancer causes & control : CCC* **17**, 137-146.

57. World Cancer Research Fund International/American Institute for Cancer Research. World Cancer Research Fund International Systematic Literature Review: The Associations between Food, Nutrition, Physical Activity, and the Riks of Prostate Cancer. 2014. http://www.wcrf.org/sites/default/files/Prostate-Cancer-SLR-2014.pdf.

58. Pelton K, Freeman MR, Solomon KR (2012) Cholesterol and prostate cancer. *Current opinion in pharmacology* **12**, 751-759.

59. Freeman MR, Solomon KR (2004) Cholesterol and prostate cancer. *Journal of cellular biochemistry* **91**, 54-69.

60. Bansal D, Undela K, D'Cruz S *et al.* (2012) Statin use and risk of prostate cancer: a meta-analysis of observational studies. *PloS one* **7**, e46691.

61. Muller SA, Holzapfel K, Seidl C *et al.* (2009) Characterization of choline uptake in prostate cancer cells following bicalutamide and docetaxel treatment. *European journal of nuclear medicine and molecular imaging* **36**, 1434-1442.

62. Ackerstaff E, Pflug BR, Nelson JB *et al.* (2001) Detection of increased choline compounds with proton nuclear magnetic resonance spectroscopy subsequent to malignant transformation of human prostatic epithelial cells. *Cancer research* **61**, 3599-3603.

63. Chester DN, Goldman JD, Ahuja JK, Moshfegh AJ. Dietary Intakes of Choline: What We Eat in America, NHANES 2007-2008. Food Surveys Research Group Dietary Data Brief No. 9. October 2011. Available at:http://ars.usda.gov/Services/docs.htm?docid=19476.

64. Freedman LS, Schatzkin A, Midthune D *et al.* (2011) Dealing with dietary measurement error in nutritional cohort studies. *J Natl Cancer Inst* **103**, 1086-1092.

65. Holmes MD, Colditz GA, Hunter DJ *et al.* (2003) Meat, fish and egg intake and risk of breast cancer. *International journal of cancer Journal international du cancer* **104**, 221-227.

66. Wirfalt E, Li C, Manjer J *et al.* (2011) Food sources of fat and sex hormone receptor status of invasive breast tumors in women of the Malmo Diet and Cancer cohort. *Nutr Cancer* **63**, 722-733.

67. Hopkins PN (1992) Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. *Am J Clin Nutr* **55**, 1060-1070.

68. Franz MJ, Bantle JP, Beebe CA *et al.* (2004) Nutrition principles and recommendations in diabetes. *Diabetes care* **27 Suppl 1**, S36-46.

Figure legends

Figure 1. Flowchart for study selection

Figure 2. Egg consumption and breast cancer risk (A) Linear dose-response meta-analyses* (B) Non-linear dose-response meta-analysis (reference=0 egg/week, $P_{non-linearity}$=0.50)**. RR=relative risk; CI=confidence interval.

Legend:

*While Adventist Health Study was included in the pooled study (Missmer, 2002), it was excluded from the egg analysis. Thus, Mills, 1989 from Adventist Health Study was included in our meta-analysis.

**Inner ticks on the x axis represent data points contributed by the studies included in the meta-analysis. Due to overlap on the level of egg intake across some data points, the number of tick marks does not correspond to the number of data points.

Figure 3. Egg consumption and ovarian cancer risk

Figure 4. Linear dose-response meta-analyses of egg consumption and prostate cancer
Legend:
*represents studies from the same cohort (Adventist Health Study)

Figure 5. Funnel plot with pseudo 95% confidence limits for linear dose-response meta-analysis of egg consumption and fatal prostate cancer