TITLE PAGE

Egg consumption and risk of cardiovascular disease in the SUN project

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Short running head: egg consumption and cardiovascular risk

The authors declare no conflict of interest.

ABSTRACT

Background/Objective: Egg consumption has been associated with the risk of cardiovascular diseases (CVD), but evidence is scarce and inconsistent. Our aim was to examine the association between egg consumption and incidence of CVD in a prospective dynamic Mediterranean cohort of 14 185 university graduates.

Subjects/Methods: Egg intake was assessed using a 136-item validated food frequency questionnaire. The baseline consumption was categorized into no consumption or <1/week, 1/week, 2-4/week, and >4/week. The presence of cardiovascular risk factors was assessed by questionnaire at baseline and the incidence of CVD was assessed using biennial assessments. The median follow-up was 6.1 years. Cox regression models were fitted to estimate multivariable-adjusted hazard ratios (HR) for CVD (myocardial infarction, revascularization procedures or stroke). Outcomes were confirmed by review of medical records.

Results: During a median follow-up of 6.1 years, 91 new confirmed cases of CVD were observed. No association was found between egg consumption and the incidence of CVD (HR=1.10, 95% CI: 0.46-2.63) for the highest vs. the lowest category of egg consumption) after adjusting for age, sex, total energy intake, adherence to the Mediterranean food pattern and other cardiovascular risk factors. Results were robust to different analytical scenarios

Conclusions: No association between egg consumption and the incidence of CVD was found in this Mediterranean cohort.

Key words: stroke, myocardial infarction, cohort, prospective study, egg consumption, dietary cholesterol

INTRODUCTION

Cardiovascular diseases (CVD) remain a major public health problem and represent the leading cause of mortality and disability-adjusted life-years lost worldwide. In fact, mortality forecasts predict that deaths due to cancer and CVD will represent more than 50 % of total mortality in 2030 (WHO, 2008).

The development of CVD is influenced by non-modifiable risk factors, such as older age, male sex, and by modifiable factors such as smoking, physical inactivity, alcohol and dietary factors (Barraj et al, 2009). Among them, the consumption of cholesterol rich-foods has been frequently invoked as a major risk factor for CVD (Qureshi et al, 2007).

Guidelines from the American Heart Association recommend healthy adults to limit their dietary cholesterol intake to less than 300 mg/day on average (Krauss et al, 2000), and individuals with high LDL cholesterol, diabetes and/or CVD to reduce their dietary intake of cholesterol, including eggs, to <200 mg/day. The National Cholesterol Education Program recommends also reducing egg yolk consumption to <2/week as a way to reduce LDL cholesterol in individuals at increased risk of coronary heart disease (NCEP, 1994).

Egg consumption has decreased in Spain from 300 units/person/year in 1987 to 191 in 2007, probably as a result of public health recommendations to restrict egg intake (Cerdeño, 2008).

Egg is a major source of dietary cholesterol with a typical egg containing on average 200 mg of cholesterol (Djousse & Gaziano, 2008). However, it is also a complete food and an inexpensive and low-calorie source of high quality protein and other important nutrients such as minerals, folate and B vitamins, which could reduce the risk of CVD (Qureshi et al, 2007).

On the other hand, current data on the effects of dietary cholesterol on serum cholesterol are limited and inconsistent, ranging from positive association to no effects (Djousse & Gaziano, 2008). Furthermore, there have been few epidemiological studies in free-living populations assessing the relationship between egg consumption and risk of CVD (Dawber et al, 1982; Fraser, 1994; Hu et al, 1999) and all of them were conducted in non-Mediterranean populations.

The aim of our study was to prospectively examine the association between egg consumption and the risk of CVD in a prospective Mediterranean cohort, the SUN (Seguimiento Universidad de Navarra) study.

SUBJECTS AND METHODS

Study population

The SUN Project is a multipurpose, dynamic cohort designed to study the prospective association of diet and other factors with various health outcomes, like hypertension, obesity, diabetes, and CVD (Martinez-Gonzalez et al, 2002).

Briefly, the recruitment of participants, 97% come from Spain, started in December 1999 and it is permanently open. All participants are university graduates, with a high educational level, and more than fifty percent of them are health professionals themselves. Information is collected using selfadministered questionnaires sent by postal mail every 2 years. The study methods have been published in detail elsewhere (Segui-Gomez et al, 2006).

For this analysis, we included participants who had already been followed up for at least 2 years. All participants who answered the baseline questionnaire before September 2007 were eligible for this analyses (n=18 249) because they have been in the cohort for sufficient time as to be able to be assessed at least after 2-year follow-up. Among them, 2 173 had not answered any of the follow-up questionnaires, and after five more mailings separated by 2 months each, they were considered lost to follow-up. We therefore retained 16 076 participants (89.3%).

We excluded those participants who self-reported baseline prevalent CVD (n=159), subjects who were outside predefined limits for total energy intake (<800 or > 4 000 kcal/d for men and <500 or > 3 500 kcal/d for women) (n=1 500), and participants with missing values for egg consumption (n=232). After exclusions, 14 185 participants remained available for the analyses.

The Institutional Review Board of the University of Navarra approved the study protocol. Voluntary completion of the baseline questionnaire was considered as informed consent.

Assessment of egg consumption

Egg consumption was assessed using a baseline semi-quantitative food-frequency questionnaire (FFQ) (136 items), repeatedly validated in Spain (Martin-Moreno et al, 1993; Fernández- Ballart et al, 2010; De la Fuente et al, 2010). Participants were asked to report how often they had consumed eggs of hen (unit of consumption was 1 egg) during the previous year. This questionnaire had nine responses for each food item, that ranged from "never or almost never" to "≥6 times/day".

We divided the participants into 4 categories based on egg consumption: no consumption or <1/week, 1/week, 2-4/week, and >4/week. Trained dietitians derived energy and nutrient intake from Spanish food composition tables, using a computer program (Mataix, 2003; Moreiras, 2009). Finally, food and nutrient intakes were adjusted for total energy intake using the residuals method (Willett et al, 1998).

Assessment of other variables

The baseline questionnaire collected also data on socio-demographic, anthropometric characteristics, lifestyle and health-related habits, family history, obstetric history for women and medical history.

Information regarding physical activity during leisure-time was gathered at baseline with a specific questionnaire previously validated in Spain (Martinez-Gonzalez et al, 2005), which assessed the time spent in 17 different activities. Each of these activities was assigned a multiple of the resting metabolic rate (MET score) (Ainsworth et al, 2000). Thus, taking also into account the weekly time spent in each activity, we obtained a value of overall weekly MET- hours for each participant.

The validity of self-reported weight, BMI leisure-time physical activity and hypertension in the SUN cohort has been previously reported (Bes-Rastrollo et al, 2005; Martinez-Gonzalez et al, 2005; Alonso et al, 2005).

Assessment of the outcome

The baseline and follow-up questionnaires asked the participants if they had received a medical diagnosis of CVD, as well as the date of diagnosis. Participants were considered to have CVD at baseline if they reported a medical diagnosis of at least one of the following conditions: myocardial infarction, stroke, coronary artery bypass or coronary revascularization procedures.

Incident cases of CVD were defined as those participants who did not have CVD at baseline and reported a physician's diagnosis of CVD in the follow-up questionnaire. An expert panel of physicians, blinded to the information on diet and risk factors, adjudicated the events by reviewing medical records applying the universal criteria for myocardial infarction (Thygesen et al, 2007) or established clinical criteria for the other outcomes.

The end-point for the present analysis was a composite outcome of incident CVD including either fatal or non-fatal myocardial infarction with or without ST elevation, coronary revascularization procedures, or fatal or non-fatal stroke.

Statistical analysis

Chi-square tests or ANOVA were used to compare proportions or means, respectively. We estimated hazard ratios (HR) and their 95% confidence intervals (CI) for the risk of incident CVD according to egg consumption at baseline using a multivariable Cox regression analysis.

Time of follow-up was defined as the time elapsed from the completion of the baseline questionnaire to the diagnosis to CVD or the date of the last follow-up questionnaire, whichever came earlier.

We fitted three multivariable-adjusted models controlling for: a) age (continuous), sex, and baseline total energy intake (continuous), b) additionally adjusting for adherence to the Mediterranean food pattern (tertiles) (Trichopoulou et al, 1995; Martínez-González et al, 2009) and c) additionally adjusting for alcohol intake (4 categories), baseline BMI (Kg/m^2, continuous), smoking status (never smoker, ex-smoker and current smoker), physical activity during leisure-time (MET-hours/week, continuous), family history of CVD years (yes/no), self-reported diabetes (yes/no), self-reported hypertension (yes/no), and self-reported hypercholesterolemia (yes/no).

We considered the lowest category of egg consumption as the reference category.

A number of sensitivity analyses were performed: a) categorizing egg consumption into 5 categories instead of four, b) assigning the value 0 of egg consumption to missing values in this variable, c) excluding those participants who had prevalent diabetes or cancer at baseline; d) excluding subjects who were following a special diet at baseline; e) including in the outcome the cases of incident angina without revascularization and f) stratified analysis by age.

Analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA). All *P* values are two-tailed and statistical significance was set at the conventional cut-off of $P < 0.05$.

RESULTS

The mean age of participants at baseline was 38.4 years (range: 20 to 90 years) and the median egg consumption was 3/week. The median follow-up was 6.1 years (mean=5.8 years).

We observed 91 confirmed cases of CVD (probable cases were excluded) during the follow-up period: 34 myocardial infarctions, 29 revascularization procedures and 28 strokes.

Table 1 presents the characteristics of the study population according to categories of egg consumption. Participants reporting higher consumption of eggs were more likely to be men, current smokers and physically more active and less likely to have history of hypertension, diabetes or hypercholesterolemia. On the other hand, participants reporting lower consumption of eggs were older and more likely to be ex-smokers.

Egg consumption was positively related to total energy intake, total fat intake, polyunsaturated, saturated and monounsaturated fat intake. Egg consumption was also directly associated with the intake of cholesterol and alcohol, but inversely associated with carbohydrate and fibre intake.

Table 2 shows the association between egg consumption and the incidence of CVD. The age-, sex- and total energy intake-adjusted HR of CVD comparing >4 eggs/week with <1 egg/week was 1.10 (95% CI, 0.45-2.52). Additional adjustments for adherence to a Mediterranean food pattern or for several cardiovascular risk factors had no impact on the HRs: 1.08 (95% CI, 0.45-2.59) and 1.10 (95% CI, 0.46-2.63) respectively.

In an analysis dividing the highest intake category (>4 eggs/week) into two additional categories (5-6/week and ≥1/day), the adjusted HR for the highest category was 0.42 (95 % CI, 0.10-1.93), when compared to the lowest category. Results did not change when excluding participants with diabetes or cancer at baseline, or when we excluded subjects following a special diet at baseline or when we assigned a value of 0 for egg consumption to participants with missing values in egg consumption (data not shown).

When we included in the definition of cardiovascular outcome the presence of angina not requiring revascularization, egg consumption (>4/week) was not associated with a higher cardiovascular risk: adjusted HR for the highest category: 0.96 (95 % CI: 0.46-2.00).

Finally, stratified analysis by median age (36 years) was not possible due to insufficient number of CVD events in the younger group. For this reason we performed an additional analysis using as cut-off point 56 years of age, which left a similar number of events in both groups (47 events among the younger participants and 44 events among the older participants). The HR for CVD for the highest egg consumption category compared to the lowest egg consumption category among participants with <=56 and >56 years were respectively HR: 0.47 (95% CI, 0.11-2.05) and 2.94 (95% CI, 0.93-9.30). The *P* value for interaction (likelihood ratio test) was 0.075.

DISCUSSION

To our knowledge, this is the first large prospective cohort that has investigated the association between egg consumption and CVD risk in a free-living Mediterranean population. We found that egg consumption was not associated with a higher risk of CVD over 6 years of follow-up among Spanish graduates, but was positively associated with smoking, and a generally unhealthier eating pattern.

Epidemiologic evidence relating directly egg consumption to coronary outcomes is relatively scarce and the dietary instruments used and the degree of control for confounding might improve (Kritchevsky, 2004). The only study that has specifically examined the relationship between egg consumption and CHD included 37 851 men and 80 082 women free of chronic diseases of the Health Professionals Follow-up Study and the Nurses Health Study (Hu et al., 1999). After adjusting for multiple confounders, those eating >7 eggs/week (as compared with those consuming <1 egg/week), had no increased risk of coronary heart disease (CHD) or stroke in either healthy men or women [RR_{men}= 1.08 (95 % CI, 0.79-1.48, RR_{women}=0.82 (95 %CI, 0.60-1.13).

In two other cohorts, eating eggs more frequently was not associated with an increase in CHD incidence (Nakamura et al, 2006) or in the risk of stroke or ischemic stroke (Qureshi et al, 2007). However, in these later studies, there was suggestive evidence for a detrimental association of higher egg consumption and increased cardiovascular risk only among diabetic subjects, among those with higher baseline blood cholesterol levels or only among older diabetics (Houston et al, 2010).

Furthermore, other findings had also been were very similar to our results (Dawber et al, 1982; Gramenzi et al, 1990; Djousse & Gaziano, 2008; Fraser, 1994; Hu et al, 1999).

Between 1 969 and 1 972, 5 133 Finnish men and women were assessed during a follow-up period of 16 years. After adjusting for age, there were no baseline differences in egg intake between those who died from CHD and those surviving to the end of the study (Knekt et al, 1994).

Finally, in the California Adventist study there was no increase in coronary risk among those with higher egg consumption compared to those consuming < 1/week (RR=1.01), but no adjustment for other dietary factors was reported (Fraser, 1999).

On the contrary, to the best of our knowledge, only three studies have reported a detrimental effect of egg consumption on incident CHD (Mann et al, 1997; Burke et al, 2007; Nettleton et al, 2008). However, the results of the Oxford Vegetarian Study (Mann et al, 1997) need to be interpreted with caution because a number of potentially important confounding factors were not considered. On the other hand, the participants in the Australian Aboriginal study (Burke et al, 2007), were clearly very different to those in our cohort.

Different mechanisms might explain the lack of relationship between egg consumption and incident CVD. First, in spite that hypercholesterolemia is a classic risk factor for CVD and that egg contributes approximately 30 % of the dietary cholesterol, a decrease in plasmatic total cholesterol levels after a reduction in dietary cholesterol of 100 mg/d is observed only in 30% of subjects.

Probably, dietary cholesterol intake is regulated by a vast number of genes (Herron & Fernandez, 2004, McNamara, 2000). However, there is evidence that some populations may benefit from low cholesterol intake, such as diabetics who may possess an abnormality in the mechanism by which they transport cholesterol (Hu et al, 1999; Qureshi et al, 2007).

Second, some dietary patterns (e.g. the Mediterranean pattern) may lead to lower CVD risk than those based only in reducing saturated fat and cholesterol intake (Kritchevsky, 2004; Martinez-Gonzalez et al, 2010; Buckland et al, 2009; Sofi et al, 2008). For example, in Japan the rates of coronary disease have continued to fall, although their food pattern includes eating egg frequently. However, the Japanese diet is relatively low in total fat and saturated fat.

Third, the relationship of egg consumption with cardiovascular outcomes depends not only on the dietary cholesterol from eggs themselves, but also on the total cholesterol intake provided by the overall food pattern (Kritchevsky & Kritchevsky, 2000). In our study, thanks to the semi-quantitative FFQ, an index of adherence to an overall food pattern and intake of energy and specific nutrients were available; therefore, we adjusted for potential dietary confounders, including the overall food pattern, and despite this, we found not association between egg consumption and incidence of CVD.

Fourth, despite egg consumption has been associated with the prevention and treatment of specific symptoms associated with chronic age-related diseases (Herron & Fernandez, 2004) and with an increased risk of type 2 diabetes in men and women (Djoussé et al, 2009), it has not shown to adversely affect other cardiovascular risk factors (i.e. blood pressure) (Qureshi et al, 2007).

Fifth, egg contained many other nutrients besides cholesterol, including unsaturated fats which may be beneficial in preventing CVD. Finally, eggs are less rich in saturated fat than many other protein foods, so they may have only a minor effect on cholesterol levels (Kritchevsky & Kritchevsky, 2000).

Our study has several methodological limitations. Our cohort is a non-representative sample of the general Spanish population since it is a young cohort formed entirely by university graduates. However, there is no biological reason to think that our results cannot be generalized to other population groups but social and/or educational background should not be ignored, and we consider that a strong internal validity is the first step to support the external validity of our findings. Besides, residual confounding might have not been totally excluded. However, we performed the analyses adjusting for the main known risk factors, and we do not consider residual confounding as a likely important cause to explain the observed results.

Another limitation is that the statistical power of our study might have been limited to detect associations between egg consumption and CVD. This limitation is especially important when considering subtypes of CVD, specific risk of CVD among diabetic participants or the potential interaction between age and egg consumption. The P value (0.075) that we found for age x egg consumption deserves confirmation in future studies.

Finally, we are probably underestimating egg consumption since we only used the units consumed, without considering eggs that might be ingredients in other foods (e.g. pastries).

The strengths of this study are that we are using data of a well-known Mediterranean cohort (the SUN study) with a relatively large sample size, a high response rate, a sufficiently long period of follow-up. The prospective design of our study could avoid the possible effect of reverse causality in the reported associations. In addition, we were able to control for a wide variety of potential confounders. Besides, the robustness of our findings in sensitivity analyses is other strength.

In conclusion, our data suggest that a higher egg consumption was not associated with any apparent increase in CVD risk among initially healthy middle-aged adults. Further research on the biological mechanisms behind the potentially increased CVD risk with frequent egg consumption is warranted. Perhaps, efforts to promote healthy lifestyles related to smoking, exercise, weight reduction and other dietary aspects are likely to have a greater impact on CHD, than those related to reduce egg consumption. Finally, further confirmation of our findings is needed through cohort studies with more statistical power and repeated measurement of diet and/or clinical trials.

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Table 1. Main characteristics of the 14 185 participants of the SUN cohort according to baseline on egg consumption (Mean and standard deviations or percentages)

	Egg consumption				
	< 1/week (n=1 073)	1/week (n=2 871)	2-4/week (n=8 696)	>4/week (n=1 545)	*P* value [a]
Age (years)	41.6	38.7	38.0	38.1	< 0.001
Baseline BMI (kg/m ^{2})	23.9	23.5	23.5	24.0	< 0.001
Baseline weight (kg)	67.8	66.3	67.0	70.0	< 0.001
Physical activity during leisure time (METs-h/week)	23.2	23.8	23.9	26.6	< 0.001
Men (%)	42.4	36.5	39.5	55.6	< 0.001
Smoking status					0.006
Ex-smoker (%)	34.1	30.6	29.0	28.1	
Current smoker (%)	22.8	22.4	21.9	23.5	
Hypertension at baseline (%)	14.1	11.1	9.8	10.7	< 0.001
Diabetes at baseline (%)	2.6	1.7	1.8	1.5	0.191
Hypercholesterolemia at baseline (%)	29.1	22.3	18.6	15.3	< 0.001
Following a special diet at baseline (%)	14.4	9.1	7.4	5.8	< 0.001
Mediterranean Diet Score (Trichopoulou *et al*)	4.5	4.4	4.3	4.0	< 0.001
Total energy intake (kcal/day)	2058	2199	2406	2642	< 0.001
Carbohydrate intake (% total energy)	45.4	44.1	43.0	42.0	< 0.001
Protein intake (% total energy)	18.2	18.3	18.1	18.0	0.048
Fat intake (% total energy)	34.0	35.6	36.9	37.9	< 0.001
Polyunsaturated fatty acid intake (% total energy)	4.9	5.0	5.3	5.4	< 0.001
Saturated fatty acid intake (% total energy)	11.2	12.1	12.6	13.2	< 0.001
Monounsaturated fatty acid intake (% total energy)	14.7	15.3	15.8	16.1	< 0.001
Cholesterol intake (mg/day)	280.8	338.9	434.5	584.0	< 0.001
Fiber intake (g/day)	30.3	28.8	26.9	24.5	< 0.001
Alcohol intake (g/day)	7.0	6.5	6.8	7.3	0.073

[a] *P* values were determined by the chi-square test or ANOVA for the difference between the groups.

Table 2. Hazard ratios (HRs) and 95 % CIs of CVD according to egg consumption.

	Egg consumption			
	< 1/week	**1week**	**2-4/week**	**>4/week**
Incident cases of CVD	11	16	53	11
Multivariable 1	1 (ref.)	0,77 (0,36-1,67)	0,99 (0,51-1,91)	1,10 (0,45-2,52)
Multivariable 2	1 (ref.)	0,78 (0,36-1,69)	0,99 (0,51-1,95)	1,08 (0,45-2,59)
Multivariable 3	1 (ref.)	0,78 (0,36-1,70)	1,00 (0,51-1,97)	1,10 (0,46-2,63)

Multivariable 1: adjusted for age (continuous), sex and total energy intake (continuous)

Multivariable 2: additionally adjusted for adherence to the Mediterranean food pattern (3 categories)

Multivariable 3: additionally adjusted for alcohol intake (4 categories), baseline BMI (Kg/m2, continuous), smoking status (3 categories), physical activity during leisure time (MET-hours/week, continuous), family history of CVD years (yes/no), self-reported diabetes (yes/no), self-reported hypertension (yes/no), self-reported hypercholesterolemia (yes/no).