TITLE PAGE

Consumo de huevo y riesgo de diabetes tipo 2 en una cohorte mediterránea: el proyecto SUN

Egg consumption and risk of type 2 diabetes in a Mediterranean cohort: the SUN project

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ABSTRACT

Introduction & Aim: The prevalence of diabetes is increasing at an alarming rate in nearly all countries. Some studies from non-Mediterranean populations suggest that higher egg consumption is associated with an increased risk of diabetes. The aim of our study was to prospectively assess the association between egg consumption and the incidence of type 2 diabetes in a large cohort of Spanish university graduates.

Methods: In this prospective cohort including 15,956 participants (mean age: 38,5 years) during 6.6 years (median), free of diabetes mellitus at baseline, egg consumption was assessed at baseline through a semi-quantitative food-frequency questionnaire repeatedly validated in Spain. Incident diabetes mellitus diagnosed by a doctor was assessed through biennial follow-up questionnaires, confirmed subsequently by medical reports or records, according to the American Diabetes Association criteria. Analyses were performed through multivariable non-conditional logistic regression.

Results: After adjustment for confounders, egg consumption was not associated with the development of diabetes mellitus, comparing the highest versus the lowest quartile of egg consumption (>4 eggs/week vs <1 egg/week): odds ratio= 0,7; 95% CI 0,3-1,7.

Conclusion: Egg consumption was not associated with the development of diabetes mellitus in this Mediterranean cohort.

KEY WORDS: Diabetes mellitus, Epidemiology, Prospective study, Egg consumption, Dietary cholesterol

ABSTRACT

Introducción y Objetivo: La prevalencia de la diabetes está aumentando a un ritmo alarmante en casi todos los países. Algunos estudios en poblaciones no mediterráneas sugieren que un mayor consumo de huevo se asocia con un mayor riesgo de diabetes. El objetivo de nuestro estudio fue evaluar prospectivamente la asociación entre el consumo de huevo y la incidencia de diabetes tipo 2 en una gran cohorte de graduados universitarios españoles.

Métodos: Un total de 15.956 participantes (edad media: 38.5 años) seguidos durante 6,6 años (mediana), y libres de la diabetes mellitus al inicio del estudio fueron incluidos en este estudio. El consumo de huevos se evaluó al inicio del estudio a través de un cuestionario semi-cuantitativo de frecuencia de alimentos repetidamente validado en España. Los casos de diabetes mellitas incidente fueron diagnosticados por un médico a través de cuestionarios de seguimiento bianuales y posteriormente confirmados por los informes médicos o registros, de acuerdo con los criterios de la American Diabetes Association. Los análisis se realizaron a través de modelos de regresión logística condicional multivariable.

Resultados: Después de ajustar por los factores de confusión, el consumo de huevo no se asoció con el desarrollo de diabetes mellitas. Odds Ratio de aquellos participantes con mayores consumos frente a los del cuartil más bajo de consumo de huevos (> 4 huevos/semana frente a <1 huevo/semana) fue 0,7, IC del 95% CI 0.3-1.7.

Conclusión: El consumo de huevos no se asoció con el desarrollo de diabetes mellitus en esta cohorte mediterránea.

PALABRAS CLAVE: Diabetes mellitus, Epidemiología, Estudio prospectivo, Consumo de huevo, Colesterol dietético

ABREVIATURAS

SUN: Seguimiento Universidad de Navarra

FFQ: Food- Frequency Questionnaire

BMI: Body Mass Index

CHS: Cardiovascular Health Study

INTRODUCTION

In recent decades the prevalence of diabetes is increasing at an alarming rate in nearly all countries and the projections for 2030 indicate a world prevalence among adults of 7,7% [1] due to the increasing prevalence of obesity and sedentary lifestyles, aging population and urbanization [2-3]. The "diabetes epidemic" remains a major public health problem and it is associated with a wide range of health complications. This chronic disease has enormous human and economic costs on the national health care systems worldwide. For example, in the United States a diabetic individual spent 2,5 times more on medical care than other individual without this condition [2].

In order to prevent this current trend on diabetes, the identification and modification of risk factors for the development of diabetes is a priority. In this context dietary characteristics (a high intake of fibre, a high intake of vegetable fat, a low intake of trans fatty acids or a moderate intake of alcohol), are a possible protective role of diabetes [4].

Diabetes may share some dietary risk factors with cardiovascular disease. It is interesting however that some dietary factors previously believed to be associated with a higher cardiovascular risk do not increase that risk in the general population, but only in diabetics. This is the case for egg consumption [5-6]. In fact, several studies from non-Mediterranean populations suggest that consumption of 1 egg/day or more is associated also with an increased risk of diabetes [7-8].

Egg is the major source of dietary cholesterol with an average of 200 mg/egg. In addition, egg is a complete food and an inexpensive low-calorie source of high quality protein [7,9] and other nutrients (minerals, folate, B, vitamins and polyunsaturated and monounsaturated fatty). Although some of these nutrients have been associated with a higher risk of diabetes, others could help to reduce this risk [7].

The American Heart Association (2000) recommended 300 mg/d of dietary cholesterol on average for healthy individuals and <200 mg/day for those with diabetes [10].

There are very few epidemiological studies in free-living populations (none in a Mediterranean population) that have examined the role of egg consumption as a complete food on the incidence of type 2 diabetes [7,8,11]. One observational study in China and another large prospective study in USA have suggested that higher egg consumption in adults during mean follow-up of 20 years respectively, was associated with an increased risk of diabetes in men and women [7-8]. However, another prospective study in older adults found no association [11].

OBJECTIVE

The aim of our study was to prospectively examine the association between egg consumption and the incidence of type 2 diabetes in a large Mediterranean cohort of university graduates.

METHODS

Study population

The SUN Project is a Spanish dynamic prospective cohort designed to assess the association between diet or other lifestyle factors and various chronic diseases like diabetes, obesity and cardiovascular diseases [12]. In short, the recruitment of participants started in 1999 and it is permanently open. All participants are university graduates and more than fifty percent of them are health professionals.

Information is collected through self-administered and mailed questionnaires every 2 years. The objectives and methods of the SUN cohort have been reported in detail elsewhere [13].

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 February 2008 were eligible for this analyses (n=21.330) 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, 3.039 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 18.291 participants (90,6%).

We excluded those participants with one or more of the following criteria: self-reported diabetes at baseline (n=312), missing values in this variable (n=91), 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=1.678), and finally we excluded participants with missing values for egg consumption (n=254). After exclusions, a final sample of 15.956 participants was used for the analysis.

The study was approved by the Human Research Ethical Committee at the University of Navarra. Voluntary completion of the first questionnaire was considered as informed consent.

Assessment of egg consumption

Egg consumption was assessed at baseline through a semi-quantitative food-frequency questionnaire (FFQ) with 136 items that has been repeatedly validated in Spain [14-16]. Participants were asked to report how often, on average, they had consumed eggs of hen (1 egg was a unit of consumption) during the previous year. The frequency of intake for each food item had nine responses, that ranged from "never or almost never" to "≥6 times/day". Besides, the methods of preparation of the eggs taken not into account.

Adherence to the Mediterranean diet was defined according to the 0 to 9 points score proposed by Trichopolou et al. (Trichopouolou *et al.*, 2003) as previously described [4].

We divided the participants into 4 categories based on the frequency of egg consumption: no consumption or <1/week, 1/week, 2-4/week, and >4/week. Nutrient intakes were calculated by trained dietitians with a computer program based on Spanish food composition tables [17-18]. Finally, food and nutrient intakes were adjusted for total energy intake using the residuals method [19].

Assessment of other variables

The baseline questionnaire also collected information on socio-demographic variables, anthropometric characteristics, medical and family history, lifestyle and health-related habits and obstetric history for women. A specific questionnaire previously validated in Spain [20] was also completed at baseline to assess the time spent during leisure-time in 17 different activities. A multiple of the resting metabolic rate (MET score) was assigned to each activity [21]. Thus, taking also into account the weekly time spent in each activity, we calculated for each participant a value of overall weekly MET- hours.

The validity of self-reported weight, BMI, leisure-time physical activity and hypertension in the SUN cohort has been previously documented in specific published studies conducted in subsamples or this cohort [20,22,23].

Assessment of diabetes

The baseline and follow-up questionnaires asked the participants whether they had received a medical diagnosis of diabetes, as well as the date of diagnosis. Participants were considered to have diabetes at baseline if they reported a medical diagnosis of diabetes or if they were on treatment with insulin and/or oral antidiabetic agents. When we observed a probable case of new onset diabetes in the follow-up questionnaires, we sent an additional questionnaire requesting more information such as date of diagnosis, symptoms of hyperglycemia, fasting glucose levels, figures of glycated hemoglobin, levels of glucose after an oral glucose tolerance test, treatment used for diabetes and type of diabetes. An expert panel of physicians, blinded to the information on diet and risk factors, adjudicated the events by reviewing medical records applying the diagnostic criteria issued by the American Diabetes Association [24].

Incident cases of diabetes were defined as those participants without a diagnosis of diabetes at baseline, who 1) reported a physician's diagnosis of diabetes in a follow-up questionnaire, 2) and completed and returned an additional questionnaire with written confirmation and medical records detailing the diagnosis, 3) and a team of medical doctors of the SUN project, blinded to the dietary exposure of the participant, reviewed their medical information and adjudicated the event as type 2 diabetes. The criteria of the American Diabetes Association were used to adjudicate these events [25]. We excluded cases of diabetes other than type 2 diabetes.

Statistical analysis

Chi-square tests or ANOVA were used to compare proportions or means, respectively. We estimated odds ratios (OR) of incident type 2 diabetes across categories of baseline egg consumption and their 95% confidence intervals (CI) for the risk of incident diabetes using multivariable logistic regression.

We fitted three multivariable-adjusted models controlling for the following baseline factors: a) age (continuous), sex, and total energy intake (continuous), b) additionally adjusting for adherence to the Mediterranean food pattern (continuous) [4,26], and c) additionally adjusting for alcohol intake (continuous), BMI (Kg/m2, continuous), smoking status (never smoker, ex-smoker and current smoker), physical activity during leisure-time (MET-hours/week, continuous), family history of diabetes (yes/no), self-reported hypercholesterolemia (yes/no), self-reported cardiovascular disease (yes/no), and self-reported hypertension (yes/no). The lowest category of egg consumption was considered 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 egg consumption to missing values (n=254) in the egg consumption variable, c) excluding those participants who had prevalent cardiovascular disease or cancer at baseline; d) excluding subjects who were following a special diet at baseline and e) including in the outcome also the incident cases of gestational diabetes (n=18).

All *P* values are two-tailed and statistical significance was set at *P* <0,05. We used SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) for all analyses.

RESULTS

The mean age of participants at baseline was 38,5 years (range: 20 to 90 years) and the median egg consumption was 3 units/week. The median follow-up was 6,6 years (mean=6,3 years). During the follow-up period, 91 new cases of diabetes were confirmed.

Baseline characteristics of the participants according to categories of egg consumption are presented in Table 1. Participants belonging to the lowest category of egg consumption were more likely to be older, female and ex-smokers and reported a higher frequency of hypertension, cardiovascular diseases, and hypercholesterolemia at baseline. These subjects presented also higher intakes of carbohydrate and fiber and a lower intake of total energy, fat, polyunsaturated and monounsaturated fatty acids, and cholesterol.

On the other hand, subjects in the highest category of egg consumption were more likely to be current smokers, physically active, and with lower adherence to the Mediterranean diet.

When we assessed the risk of diabetes according to the baseline consumption of egg after adjustment for age, sex, total energy intake, adherence to a Mediterranean food pattern and for several diabetes risk factors (Table 2), higher egg consumption was non-significantly associated with a lower risk for the development of diabetes. The OR for diabetes comparing participants consuming >4 eggs/week versus those consuming <1 egg/week was 0,7 (95% CI, 0,3-1,7).

However, in the multivariable 2 model, adjusted for age, sex, total energy intake and for adherence to the Mediterranean food pattern, consumption of 2-4 eggs per week (but not > 4 eggs/week) was associated with lower risk of diabetes (HR 0,5; 95% CI: 0,3, 0,9) versus consuming <1 egg/week.

When we performed the sensitivity analyses dividing the highest intake category (>4 eggs/week) into two additional categories (5-6/week and ≥1/day) the ORs were: 0.5 (95% CI, 0,2-1,5) and 1.2 (95% CI, 0,4-3,2). When we excluded persons with cancer or cardiovascular diseases at baseline or subjects following a special diet at baseline, we observed similar results (data not shown). Finally when we repeated the analysis assigning a value of 0 for egg consumption to participants with missing values in egg consumption or when incident cases of gestational diabetes were included in the definition of the outcome, the results were essentially the same (data not shown).

DISCUSSION

To our knowledge, no previous study has examined prospectively the association of egg consumption and risk of diabetes in a large free-living Mediterranean population. However, we found in a previous publication on this same cohort no association between egg consumption and the incidence of cardiovascular disease, a factor risk of diabetes [27].

Our research suggests that egg consumption was not associated with the incidence of type 2 diabetes after controlling for age, gender and for the main known risk factors of this disease, including alcohol intake, baseline BMI, smoking status, physical activity during leisure-time,

family history of diabetes, self-reported hypercholesterolemia, self-reported cardiovascular disease and self-reported hypertension. An important strength of our assessment is that we also controlled for the overall dietary pattern.

These results are in line with those from the Cardiovascular Health Study (CHS). In that prospective cohort of 3898 men and women there was no association between egg consumption or dietary cholesterol and incident diabetes [11]. However, some differences between the two studies merit some considerations. The participants of the CHS were ≥ 65 years old and their median egg consumption was < 1 egg/week. On the contrary, in our study average age was 38,5 years and mean consumption was 3 eggs/week.

However, our results are not in agreement with findings from two studies that have shown that eating eggs more frequently was associated with an increase in the risk of developing type 2 diabetes. In a large prospective study, high levels of egg consumption (at least one egg/day) was associated with an increased risk of type 2 diabetes in 20.073 men from the Physicians' Health Study and 36.295 women from the Women's Health Study [7]. Thus, eating >7 eggs/week (as compared with those consuming <1 egg/week), had a hazard ratio for diabetes of 1,58 (1,25-2,01) among men and 1,77 (1,28-2,43) among women after adjusting for multiple confounders.

In other study in Chinese population, egg consumption was also positively associated with the risk of diabetes, particularly in women, independently of traditional risk factors for this disease [8]. The OR (95 % CI) of diabetes associated with frequency of consumption ≥1 eggs/day compared to <2 eggs/week was 2,28 (1,15-4,54) (P for trend 0,029).

The inconsistencies observed between our results and the Chinese and American findings may be related to differences in characteristics of participants (e.g. the mean age was > 53 years in the American study), in the dietary pattern or in the different follow-up of participants (20 years in the study of Djuossé and 6,6 years in our study). However, even our point estimate for the association was under the null value. In any case, it is possible that egg consumption might be associated with higher diabetes risk only at levels of consumption above 5 eggs/week and the

small number of new cases at those levels of consumption in our cohort did not allow us to observe that association.

Although the potential biological mechanisms by which high egg consumption might influence the risk of type 2 diabetes are largely unknown (eggs are very poor in carbohydrates), there are different explanations that might account for the lack of association in our study. First, it could be thought that a low biological plausibility exists to support that a two years exposure might not be sufficient for revealing an association between a dietary habit and diabetes development. For this reason, we excluded participants with a follow-up period shorter than 4 years and repeated the analyses. The results showed a significanty association between eating 2-4 eggs/week and a lower risk for diabetes, compared with consuming <1 egg/week: the ORs for that category was 0,50 (95 % CI, 0,26-0,95). Second, the individual response to dietary cholesterol is due to variability in genetic and nongenetic factors [9,28,29]. Thus, a reduction of 100 mg/d in cholesterol intake only decreases the plasmatic total cholesterol levels in 30% of subjects. Therefore, the genetic background or some environmental factors of a specific population might explain different responses to dietary cholesterol intake regarding the risk of type 2 diabetes. Third, epidemiological evidence suggests that adherence to the Mediterranean dietary pattern is associated with a significant reduction in the risk of developing type 2 diabetes [4,30-33] or cardiovascular disease [34]. In particular, some specific components of Mediterranean diet (virgin olive oil, fruits, vegetables, nuts or whole grains) are likely to protect against insulin resistance and the metabolic syndrome [4,30-33]. It could therefore happen that our participants, with moderate adherence to the Mediterranean dietary pattern, might be protected for diabetes mellitus, in front of a potential cause of diabetes like egg consumption. For example, it is common in the Mediterranean area to use abundant olive oil as culinary fat or for dressing various dishes [35-36]. Thus, for example one of the most delicious dishes of our cuisine is the Spanish potato omelet. Fourth, some authors have suggested that total dietary cholesterol might be related to incident diabetes [11]. Since we did not take into account sources of cholesterol other than egg consumption, these other sources might act as potential confounders in our analysis. However, we assessed the risk of diabetes according to baseline dietary cholesterol intake categorized in quartiles, and we found no association. And finally, in spite that eggs contain saturated fat and cholesterol that might increase the development of type 2

diabetes [8], they also contain other potentially beneficial nutrients, such as monounsaturated and polyunsaturated fatty acids that might prevent this disease [37-39].

Our study has some limitations. The number of incident cases of diabetes was small and in consequence the statistical power might have been limited to detect associations between eating eggs more frequently and an increase in type 2 diabetes. However, the number of new cases of diabetes in a young cohort (mean age is 38,5 years) with high absolute levels of consumption of typical foods in a Mediterranean diet [4,40], is expected to be low. Another limitation is related to the generalizability of our findings in a young cohort of university graduates that is a non-representative sample of the general Spanish population. However, there is no biological argument to suppose that their dietary behaviors, including egg consumption, could have a different influence on the incidence of diabetes due to socioeconomic and/or educational backgrounds. Indeed, a strong internal validity, related to the quality of the information provided by highly educated subjects, high retention rate, adjustment for potential confounders, and confirmation of incident cases using medical documentation, is the first step to support the external validity of our results.

As it might happen in any observational study, residual confounding cannot be totally excluded. However, we adjusted for known and suspected confounders, and we consider that residual confounding is unlikely.

Another potential limitation might be related to the potential measurement error in the FFQ that we used, which provides only subjective information. However, our FFQ has been repeatedly validated in Spain [14-16].

Finally, egg consumption might be underestimated since we only have considered the units of this food consumed, but not eggs or yolk contained in other products (e.g. pastries).

On the other hand, the prospective design of the study, the large sample size, a high response rate, long duration of follow-up, the control for a wide variety of potential confounders and the robustness of the findings in sensitivity analyses are major strengths of our study.

CONCLUSION

In conclusion, our data suggest that higher egg consumption was not associated with elevated risk for type 2 diabetes. Future studies on potential biological mechanisms that may explain the association between frequent egg consumption and type 2 diabetes are warranted. Finally, confirmation of these findings in other Mediterranean population is needed.

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Table 1. Baseline main characteristics of the 15.956 participants of the SUN cohort according to egg consumption (mean and standard deviations or percentages)

	Egg consumption			
	< 1 egg/week n=1.227	**1 egg/week n=3.309**	**2-4 eggs/week n=9.761**	**>4 eggs/week n=1.659**
Age (years)	41,8 (13,5)	38,7 (12,0)	38,0 (11,8)	38,0 (12,0)
Baseline BMI (kg/m ^{2})	23,9 (3,8)	23,4 (3,5)	23,4 (3,4)	24,0 (3,4)
Baseline weight (kg)	68,0 (14,2)	66,33(13,5)	66,9 (13,3)	70,2 (13,6)
Physical activity during leisure time (METs-h/week)	20,3 (21,0)	20,78(22,5)	21,1 (21,6	22,7 (24,6)
Men (%)	42,3	36,2	39,0	55,6
Smoking status				
Ex-smoker (%)	35,1	30,7	29,0	28,0
Current smoker (%)	22,2	22,1	21,4	23,9
Hypertension at baseline (%)	14,7	11,2	9,7	10,3
ECV at baseline (%)	2,7	0,9	0,9	1,1
Hypercholesterolemia at baseline (%)	28,0	20,9	15,2	11,2
Following a special diet at baseline (%)	13,9	8,7	6,8	5,2
Mediterranean Diet Score (Trichopoulou *et al*)	4,4 (1,8)	4,3 (1,8)	4,2 (1,8)	3,9 (1,8)
Total energy intake (kcal/day)	2.054 (634)	2.190 (601)	2.410 (586)	2.637 (587)
Carbohydrate intake (% total energy)	45,3 (8,4)	44,1 (7,6)	43,1 (7,1)	42,0 (7,1)
Protein intake (% total energy)	18,2 (3,7)	18,3 (3,4)	18,1 (3,1)	18,0 (2,9)
Fat intake (% total energy)	34,2 (7,4)	35,6 (6,6)	36,8 (6,3)	37,9 (6,2)
Polyunsaturated fatty acid intake (% total	4,9 (1,7)	5,0 (1,5	5,2 (1,5)	5,4 (1,5)

energy)				
Saturated fatty acid intake (% total energy)	11,3 (3,7)	12,2(3,3)	12,6 (3,0)	13,2 (3,1)
Monounsaturated fatty acid intake (% total energy)	14,8 (4,1)	15,3 (3,7)	15,8 (3,6)	16,1 (3,5)
Cholesterol intake (mg/day)	283,2 (112,3)	337,6 (124,4)	433,7 (121,5)	583,2 (166,4)
Fiber intake (g/day)	30,3 (12,3)	28,6 (10,8)	27,0 (10,1)	24,3 (10,0)
Alcohol intake (g/day)	7,1 (9,6)	6,5 (9,4)	6,7 (10,1)	7,5 (11,8)

Table 2. Odds Ratios (ORs) for incident diabetes according to categories of egg consumption in the SUN cohort (n=15.956)

	Egg consumption			
	< 1/week	**1week**	**2-4/week**	**>4/week**
n	1.227	3.309	9.761	1.659
Incident cases of diabetes	15	22	44	10
Crude model	1 (ref.)	0,5 (0,3-1,1)	0,4 (0,2-0,7)**	0,5 (0,2-1,1)
Multivariable 1	1 (ref.)	0,7 (0,4-1,5)	0,5 (0,3-0,9)*	0,6 (0,2-1,3)
Multivariable 2	1 (ref.)	0,7 (0,4-1,4)	0,5 (0,3-0,9)*	0,5 (0,2-1,2)
Multivariable 3	1 (ref.)	0,9 (0,4-1,8)	0,6 (0,3-1,2)	0,7 (0,3-1,7)

*p<0,05 ** p<0,01

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

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

Multivariable 3: additionally adjusted for alcohol intake, baseline BMI, smoking status, physical activity during leisure time, family history of diabetes, self-reported ECV, self-reported hypertension, self-reported hypercholesterolemia.