



Trabajo Original

Epidemiología y dietética

Egg consumption and dyslipidemia in a Mediterranean cohort

Consumo de huevo y dislipidemia en una cohorte mediterránea

Zenaida Vázquez-Ruiz¹, Carmen de la Fuente-Arrillaga¹, Maira Bes-Rastrollo¹, Itziar Zazpe², Susana Santiago², Cristina Razquin¹, Estefanía Toledo¹ and Miguel Ángel Martínez-González^{1,3}

¹Department of Preventive Medicine and Public Health. School of Medicine. Clínica Universidad de Navarra. Universidad de Navarra. Centro de Investigación Biomédica en Red Área de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN). IdiSNA, Navarra Institute for Health Research. Pamplona, Navarra. Spain. ²Department of Nutrition and Food Sciences, Physiology and Toxicology. School of Pharmacy. Universidad de Navarra. IdiSNA, Navarra Institute for Health Research. Pamplona, Navarra. Spain. ³Department of Nutrition. Harvard TH Chan School of Public Health. Boston, USA

Abstract

Introduction and objectives: Our aim was to prospectively evaluate the association between egg consumption and dyslipidemia in a Mediterranean cohort.

Methods: We followed-up 13,104 Spanish university graduates for a mean period of 8 years. Dietary habits at baseline were assessed using a validated semi-quantitative 136-item food-frequency questionnaire. Self-reported blood concentrations of total cholesterol, high-density lipoproteins cholesterol (HDL-c) and triglycerides were evaluated according to categories of egg consumption after 6 and 8 years of follow-up. We also assessed the association between baseline egg consumption and the incidence of hypercholesterolemia, low HDL-c concentrations and hypertriglyceridemia during follow-up.

Results: We observed a significant inverse association for intermediate levels of egg consumption (2 to 4 eggs/week vs. less than 1 egg/week) and hypertriglyceridemia with OR = 0.71 (95% confidence interval [CI]: 0.54 to 0.93, $p < 0.05$) in the multivariable-adjusted model. Using HDL-c values after 8-year follow-up, we found an association between higher egg consumption and lower HDL-c levels (p for trend = 0.02) with an adjusted difference of -4.01 mg/dl (-7.42 to -0.61) for > 4 vs. < 1 egg/week. Lower means of triglycerides were found in each of the three upper categories of egg consumption compared to the lowest category (< 1 egg/week) with significant results for some of these categories both after 6 and 8 year follow-up.

Conclusions: Our data do not support that higher egg consumption was associated with abnormal blood levels of total cholesterol or triglycerides; an inverse association with HDL-c as a quantitative variable was found only in one of our analyses.

Key words:

Egg consumption.
Cohort. Cholesterol.
HDL-c. Triglycerides.
Dyslipidemia.

Resumen

Introducción y objetivos: evaluar prospectivamente la asociación entre el consumo de huevo y el riesgo de dislipidemia en una cohorte mediterránea.

Métodos: se siguieron 13.104 graduados universitarios españoles durante un periodo medio de 8 años. La dieta se evaluó al inicio utilizando un cuestionario semicuantitativo de frecuencia de consumo de alimentos repetidamente validado. Las concentraciones sanguíneas de colesterol total, lipoproteínas de alta densidad (HDL-c) y triglicéridos autorreferidas fueron evaluadas según categorías de consumo de huevo tras 6 y 8 años de seguimiento. También se evaluó la asociación entre el consumo basal de huevo y la incidencia de hipercolesterolemia, concentraciones bajas de HDL-c e hipertrigliceridemia durante el seguimiento.

Resultados: se observó una asociación entre los niveles intermedios de consumo de huevo (2-4 unidades/semana frente a < 1 unidad/semana) y menor riesgo de hipertrigliceridemia con OR = 0,71 (intervalo de confianza del 95% [IC]: 0,54 a 0,93, $p < 0,05$) en el modelo más ajustado. Tras 8 años de seguimiento, encontramos una asociación entre un mayor consumo de huevo y menores niveles de HDL-c (p tendencia lineal = 0,02) con una diferencia ajustada de $-4,01$ mg/dl ($-7,42$ a $-0,61$) para > 4 vs. < 1 unidad/semana. Se encontraron menores concentraciones de triglicéridos en las tres categorías superiores de consumo de huevo en comparación con la inferior con resultados significativos para algunas de estas categorías después de 6 y 8 años de seguimiento.

Conclusiones: un mayor consumo de huevo no se asoció con niveles anormales de colesterol total o triglicéridos; se encontró una asociación inversa con HDL-c como variable cuantitativa solo en uno de nuestros análisis.

Palabras clave:

Consumo de huevo.
Cohorte. Colesterol.
HDL-c. Triglicéridos.
Dislipidemia.

Received: 15/06/2017 • Accepted: 19/07/2017

Vázquez-Ruiz Z, de la Fuente-Arrillaga C, Bes-Rastrollo M, Zazpe I, Santiago S, Razquin C, Toledo E, Martínez-González MA. Egg consumption and dyslipidemia in a Mediterranean cohort. *Nutr Hosp* 2018;35:153-161

DOI: <http://dx.doi.org/10.20960/nh.1347>

Correspondence:

Miguel Ángel Martínez-González. Department of Preventive Medicine and Public Health. School of Medicine. Clínica Universidad de Navarra. Universidad de Navarra. c/ Irunlarrea, 1. 31080 Pamplona, Navarra. Spain
e-mail: mamartinez@unav.es

INTRODUCTION

Limiting egg consumption to avoid dyslipidemia has been customarily recommended, to reduce the risk of cardiovascular disease (CVD) (1). However, dietary cholesterol is known to have only a limited influence on total serum cholesterol and in low-density lipoprotein cholesterol (LDL-c) levels. Moreover, increasing egg consumption has been associated with beneficial moderate elevations in high-density lipoprotein cholesterol (HDL-c) (2) and even sometimes with beneficial overall serum lipid profiles when the size and number of lipoprotein particles were also taken into account (3-5). Available meta-analyses do not find any association between egg consumption and the risk of CVD (2,6,7). Almost all previous guidelines recommended that cholesterol intake should be limited to no more than 300 mg/day, but the more recent and comprehensive evidence showed no demonstrable harms associated to egg consumption or to a greater dietary cholesterol intake (2,6-8). It is widely assumed that dietary "cholesterol is not considered a nutrient of concern for overconsumption" (9). However, this conclusion has raised some debate (10) given that eggs are one of the major sources of dietary cholesterol, one unit (60 g) provides approximately 230 mg of cholesterol. Hence, the effect of egg consumption on serum lipid levels remains controversial (2,8,11-13), particularly on serum levels of total cholesterol and LDL-c. Some studies relating egg consumption to lipid levels used extreme and unrealistic categories of exposure, or classified subjects at post-hoc in subgroups according to whether or not they were "responders" (8). Most studies did not take into account the effect of ethnicity, dietary confounders and nutritional effect modifiers. Interestingly, some aspects of the overall dietary pattern may differentially affect the serum lipid response to egg consumption (14) and this response has shown marked between-subject variation and between-study heterogeneity (2) which is likely to depend, at least partially, on the overall diet. In this context, studies on different ethnic groups are important. The Mediterranean diet has been proposed by the Dietary Guidelines Advisory Committee as a healthy dietary pattern with strong and robust evidence of protection and its global use is currently on the rise (15). It is therefore interesting to assess the lipid responses to egg consumption in cohorts of free-living subjects who live in Mediterranean areas. We evaluated the association between the frequency of egg consumption and the incidence of hypercholesterolemia, low levels of HDL-c or high values of triglycerides in a large cohort of Spanish university graduates, the SUN (Seguimiento to Universidad de Navarra) cohort.

METHODS

The SUN project is a prospective and dynamic cohort. The design, objectives and methods have been previously published (16,17). More than 50% of participants in the SUN cohort are health professionals themselves. The recruitment started in December 1999 and it is permanently open (dynamic design). The answer of the first questionnaire is considered as provision of informed consent.

Sociodemographic variables, lifestyles and medically-diagnosed diseases were collected through a baseline questionnaire at the time of recruitment. The protocol for the SUN cohort was approved by the Institutional Review Board at the University of Navarra. We inquired about serum total cholesterol levels and about the criteria for metabolic syndrome (MetS), including HDL-c, TG and fasting blood glucose levels, in the 6-year and 8-year follow-up questionnaires. This self-reported information provided by highly educated participants has previously been validated (18,19). We also inquired about new medical diagnoses of hypercholesterolemia in all follow-up questionnaires (after 2, 4, 6, 8, 10, 12 and 14-year follow-up). We considered the follow-up time as the period between recruitment (reception of the baseline questionnaire) and the date of the last follow-up questionnaire, the date of a new diagnosis of hypercholesterolemia or death, whichever came first. From a total of 22,175 participants in the SUN cohort (Fig. 1), we excluded those participants with less than 6 years and 9 months of follow up ($n = 3,072$), and those who were lost to follow up or had missing values in the variables of interest ($n = 4,208$). We also excluded participants taking lipid-lowering drugs at the time of inclusion in the study ($n = 417$) and those who were outside pre-defined values for total energy intake (< 800 or $> 4,000$ kcal/day for men and < 500 or $> 3,500$ kcal/day for women) ($n = 1,374$) (20). The 13,104 remaining participants were available for different analyses. For the assessment of the association between egg consumption and follow-up values of total blood cholesterol, we excluded participants with baseline values of total blood cholesterol above 200 mg/dl ($n = 3,017$) and those who did not report their total blood cholesterol data at 6 or 8 years of follow up ($n = 3,564$). Therefore, we included in this analysis 6,523 participants without baseline hypercholesterolemia and with available data for blood total cholesterol at follow-up. To assess the association between egg consumption and HDL-c levels, we excluded participants with prevalent low levels of HDL-c, below 40 mg/dl in men or below 50 mg/dl in women ($n = 903$) and those who had been in the cohort for less than 6 years (they could not provide lipid values at 6 or 8 years of follow-up) or did not report their levels of HDL-c at 6 or 8 years of follow-up ($n = 6,002$). The available subsample for the analysis on HDL-c was 6,199 participants. Finally, to study the prospective association between egg consumption and incidence of hypertriglyceridemia we excluded from the initial sample (13,104 participants), participants with triglycerides levels above 150 mg/dl at baseline ($n = 739$) and those who did not report their data of triglycerides blood levels at 6 or 8 years ($n = 5,188$). The final subsample for assessing the association between egg consumption and hypertriglyceridemia was 7,177.

DIETARY ASSESSMENT

Baseline egg consumption was assessed with a semi-quantitative 136-item food frequency questionnaire repeatedly validated in Spain (21,22). The amount of egg contained in other products (pastries or sauces) was not considered and the method for the culinary preparation of eggs was not taken into account.

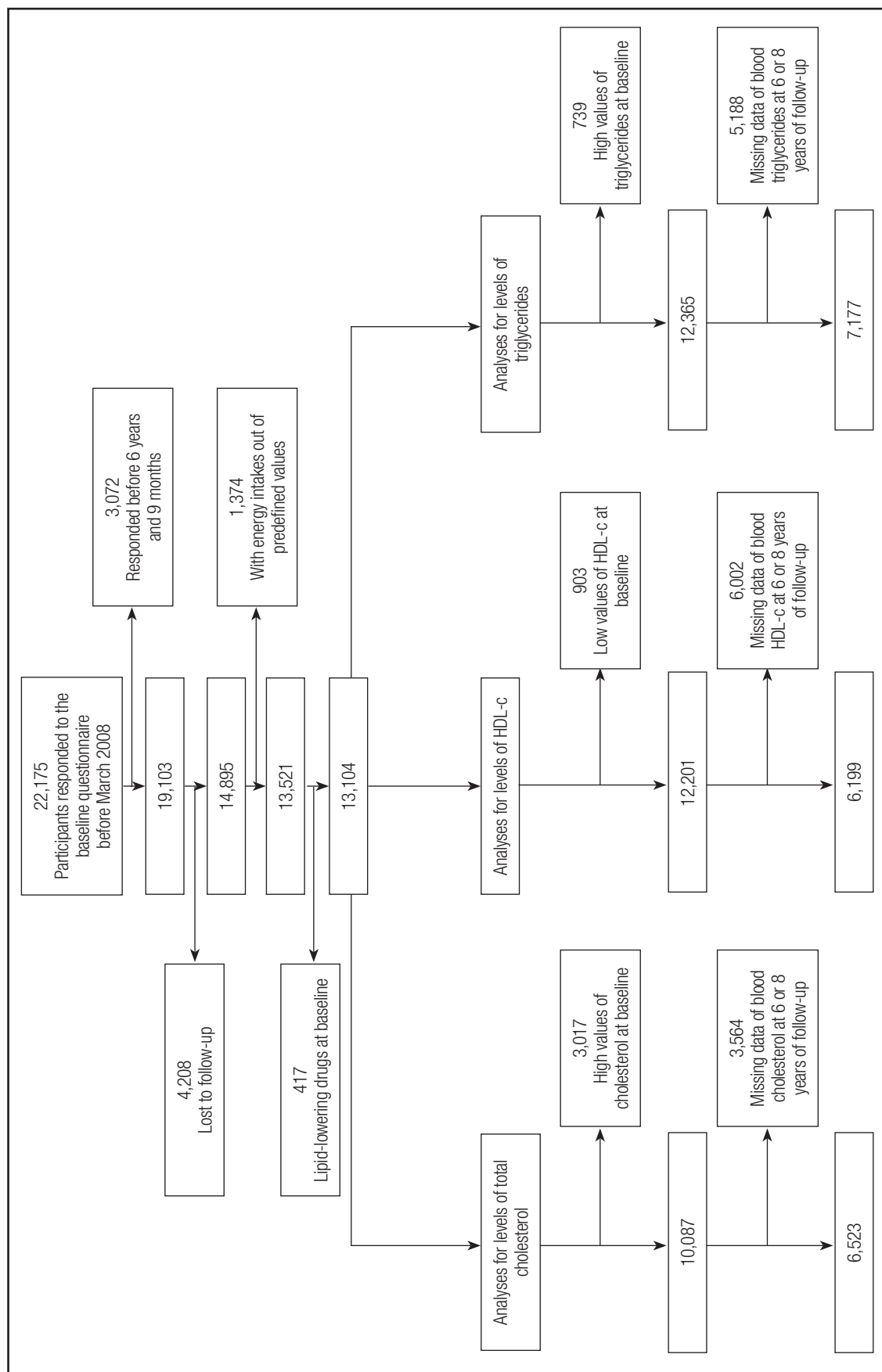


Figure 1. Flowchart of participants in the SUN Project.

Response options were nine categories: from never or almost never to more than six times a day. We assigned the value 0 egg consumption to participants with missing values in the corresponding item of the food frequency questionnaire (77 for total cholesterol analysis, 71 for HDL-c analysis and 86 for triglycerides analysis). Nutrient intake was calculated and trained dietitians updated the data bank using the latest Spanish food composition tables (23,24).

EXPOSURE ASSESSMENT AND OTHER VARIABLES

The initial questionnaire included questions on medical history and conditions including dyslipidemia, family history of cardiovascular disease, prevalent diagnoses of hypertension or diabetes, lifestyles (smoking habits, physical activity and alcohol consumption) and sociodemographic variables. Participants were classified according to their smoking habits as never smokers, current smokers and former smokers. In addition, physical activity was assessed at baseline with a validated 17-item questionnaire (25). The index of metabolic equivalent task hours per week (METs-h/week) was computed by using the time spent engaging in 17 activities and multiplying the time spent by the resting metabolic rate (MET-score) specific for each activity. The METs-h/week for all activities were combined to obtain a value of total METs-h/week (26). The validity of self-reported weight, BMI, leisure time physical activity and hypertension in the SUN cohort was assessed in specific validation studies (25,27,28). Adherence to the Mediterranean diet was defined according to the 9-item score proposed by Trichopoulou et al. (29).

ASSESSMENT OF INCIDENT HYPERCHOLESTEROLEMIA, HYPERTRIGLYCERIDEMIA OR LOW LEVELS OF HDL-C

The baseline questionnaire included a question on whether the participant had previously received a medical diagnosis of hypercholesterolemia (≥ 240 mg/dl) (30) or hypertriglyceridemia (triglycerides ≥ 150 mg/dl) and the age of these diagnoses. Baseline HDL-c levels also were self-reported in a period of less than 5 years previous to the baseline questionnaire. Information on the concentrations of total serum cholesterol, triglycerides and HDL-c was collected at 6 and 8 years of follow-up. Participants reported their results obtained from their blood test and medical check-ups that they routinely undergo in Spain at no cost using the clinical services of the National Health System or from their Occupational medical services. Information about LDL-c also was collected in the 6- and 8-year follow-up questionnaires, but participants were not asked at baseline about their blood concentrations of LDL-c, therefore we could not exclude those who were prevalent cases at baseline, and due to this reason we did not include in this work any analysis relating egg consumption to serum levels of LDL-c. We assumed that participants had elevated baseline cholesterol (prevalent hypercholesterolemia) if they reported having been diagnosed of hypercholesterolemia by a medical doctor or if they

were under usual treatment with lipid-lowering drugs. Incident cases of hypercholesterolemia were defined as those participants without any baseline diagnosis of hypercholesterolemia and without any treatment with lipid-lowering drugs at baseline who subsequently reported a medical diagnosis of high blood cholesterol during follow-up.

STATISTICAL ANALYSIS

We divided participants according to their usual egg consumption in four categories: < 1 egg/week; 1 egg/week; 2-4 eggs/week and > 4 eggs/week. Odds ratios (OR) and 95% confidence intervals (CI) for incident hypercholesterolemia were estimated for the three upper categories of egg consumption using multivariate logistic regression models adjusted for potential confounders. The lowest category of egg consumption was considered as the reference category. We adjusted for the following confounding factors: age (continuous), sex, total energy intake (continuous), quartiles of body mass index (BMI), smoking (never smoker, former smoker and current smoker), physical activity during leisure time (METs-hours/week, continuous), adherence to the Mediterranean dietary pattern (continuous), alcohol intake (continuous), family history of CVD (yes/no), diabetes at baseline (yes/no) and prevalent hypertension (yes/no). Multiple linear regression models were used to evaluate the multivariable-adjusted differences in total cholesterol, HDL-c and triglycerides between each of the 3 upper categories of egg consumption and the lowest category, adjusting for the previously mentioned potential confounding factors. For triglycerides blood concentrations analyses we additionally adjusted for carbohydrate intake as an independent covariate in the regression models. The analyses were run with Stata software version 12 (Stata Corp). Statistical significance was set at the conventional cut-off of $p < 0.05$.

RESULTS

Baseline characteristics of participants according to their baseline categories of egg consumption in the subsample of total cholesterol analysis are presented in table I. The mean age of participants was 38.4 years (range 20-89) and their median egg consumption was 2.79 eggs/week. Table II shows the ORs for the association between baseline categories of egg consumption and the diagnosis of high levels of total blood cholesterol, low concentrations of HDL-c or high concentrations of triglycerides during follow-up (either at 6 or 8 years of follow up). We classified also as cases of incident hypercholesterolemia those participants who reported values of total cholesterol above 240 mg/dl in the 6-year or 8-year follow-up questionnaire. We observed an inverse association between egg consumption and high blood cholesterol in the crude model with a significant linear trend ($p = 0.03$). The comparison between extreme categories also showed, but only in the crude model, a significant inverse association (OR: 0.71; 95% CI: 0.52 to 0.96, $p < 0.05$). However, when we adjusted for age, sex and total energy intake, the inverse association weakened and the statistical significance was lost.

Neither the observed ORs for each of the 3 upper categories, nor the linear trend tests showed any significant association between egg consumption and a medical diagnosis of low HDL-c. For the association between egg consumption and the incidence of hypertriglyceridemia (values of blood levels of triglycerides > 150 ml/dl) we observed a significant *inverse* association only for intermediate levels of egg consumption (2 to 4 eggs/week vs. less than 1 egg/week) with OR: 0.71 (95% CI: 0.54 to 0.93, $p < 0.05$) in the multivariable-adjusted model. We additionally adjusted for carbohydrate intake (g/day) and we observed similar results (OR: 0.71; 95% CI: 0.54 to 0.94, $p < 0.05$). However we did not observe any statistically significant linear trend relating higher egg consumption to lower risk of hypertriglyceridemia.

We also treated the lipid levels as continuous variables and applied multiple linear regression models. Tables III and IV show the regression coefficients that represent the adjusted differences in mean levels of lipids between each of the three upper categories of egg consumption *versus* the lowest category (< 1 egg/week) which was always the reference category. Table III presents differences in means after 6-years of follow-up and table IV, after 8-years of follow-up. For average total cholesterol we observed small and non-significant differences between groups of egg consumption. Only in the crude model, higher egg consumption was associated with lower means of HDL-c blood levels (p for trend = 0.004).

Table I. Baseline characteristics of participants of the SUN cohort classified according to categories of egg consumption in the subsample of total cholesterol analyses (n = 6,523)*

	Categories of egg consumption			
	1	2	3	4
	< 1 egg /week	1 egg/week	2-4 eggs/ week	> 4 eggs/week
n	473	1,271	4,071	708
Sex (% female)	67	69.8	66.1	46.6
Age (years)	39.4 (12.01)	37.7 (11.05)	37.7 (11.08)	38.5 (11.3)
BMI at baseline (kg/m ²)	23.3 (3.39)	23.1 (3.39)	23.1 (3.22)	23.8 (3.22)
Current smokers	20.1	21.5	20.8	20.2
Former smokers	32.14	29.35	27.29	27.82
Family history of cardiovascular disease	14.4	12.51	12.1	12.3
Prevalent diabetes	1.48	1.89	1.5	1.50
Prevalent hypertension	6.98	5.59	5	6.80
Lipid-lowering drugs at follow-up	5.5	4.2	2.8	1.9
Total energy intake (kcal/day)	2,043 (662)	2,206 (597)	2,414 (573)	2,630 (576)
Carbohydrates intake (g/day)	235 (94.4)	245 (84.1)	262 (80.6)	280 (83.5)
Protein intake (g/day)	86.9 (32.2)	98.4 (26.7)	107.7 (25.6)	116 (25.3)
Fat intake (g/day)	79.4 (31.1)	87.9 (29.2)	98.9 (28.9)	110.3 (29.6)
Saturated fatty acids intake (g/day)	26.2 (12.1)	30 (11.6)	33.71 (11.2)	38.3 (11.9)
Monounsaturated fatty acids intake (g/day)	34.5 (14.6)	37.7 (13.8)	42.6 (13.9)	47 (14.1)
Polyunsaturated fatty acids intake (g/day)	11.4 (5.78)	12.3 (5.24)	14.1 (5.59)	15.9 (5.77)
Trans fatty acids intake (g/day)	0.80 (0.60)	0.91 (0.54)	1 (0.53)	1.14 (0.59)
Omega-3 fatty acids (g/day)	2.40 (1.30)	2.51 (1.20)	2.70 (1.20)	2.80 (1.20)
Omega-6 fatty acids (g/day)	9.12 (5.20)	9.81 (4.89)	11.47 (5.34)	13.17 (5.39)
Cholesterol intake (mg/day)	275 (123)	336 (120.5)	431 (116.9)	574 (152.1)
Alcohol consumption (g/day)	5.6 (7.8)	5.6 (8.2)	6.2 (9.2)	7.6 (12)
Whole-fat dairy products consumption (g/day)	154.4 (171.2)	184.6 (192.6)	211 (196.3)	263 (229)
Low-fat dairy products consumption (g/day)	198.9 (247)	231 (240)	228 (244)	189.5 (227)
Wine consumption (ml/day)	29.42 (60.12)	26.06 (55.12)	29.45 (63.8)	39.85 (89.87)
Fibre intake (g/day)	27.4 (14.3)	28 (13.9)	28.6 (11.9)	27.5 (11.2)
Physical activity (METs-h/week)	20.1 (22.8)	21.7 (23.8)	21.4 (21.8)	23.2 (25.8)

*Baseline characteristics of participants are presented as means \pm SDs for quantitative variables and as n (%) for categorical variables.

Table II. Odds Ratios (ORs) for incidence of high total cholesterol levels (TC), low high-density lipoproteins (HDL-c) levels and high triglycerides (TG) levels according to categories of egg consumption in the SUN cohort at 6 and 8 years of follow-up

	Categories of egg consumption				p for trend
	1	2	3	4	
	< 1 egg /week	1 egg/week	2-4 eggs/week	> 4 eggs/week	
<i>High TC</i>	<i>n = 473</i>	<i>n = 1,271</i>	<i>n = 4,071</i>	<i>n = 708</i>	
Incident cases n (%)	94 (19.87%)	228 (17.94%)	694 (17.05%)	106 (14.97%)	
Crude model	1 (ref.)	0.88 (0.67 to 1.15)	0.83 (0.65 to 1.05)	0.71 (0.52 to 0.96)*	0.03*
Multivariable 1	1 (ref.)	0.94 (0.72 to 1.24)	0.91 (0.71 to 1.16)	0.77 (0.56 to 1.06)	0.11
Multivariable 2	1 (ref.)	0.92 (0.70 to 1.21)	0.88 (0.69 to 1.13)	0.75 (0.55 to 1.04)	0.08
<i>Low HDL-c</i>	<i>n = 501</i>	<i>n = 1,255</i>	<i>n = 3,805</i>	<i>n = 638</i>	
Incident cases n (%)	85 (16.97%)	194 (15.46%)	535 (14.06%)	98 (15.36%)	
Crude model	1 (ref.)	0.89 (0.68 to 1.18)	0.80 (0.62 to 1.03)	0.89 (0.65 to 1.22)	0.31
Multivariable 1	1 (ref.)	0.91 (0.69 to 1.21)	0.84 (0.65 to 1.08)	0.96 (0.69 to 1.33)	0.67
Multivariable 2	1 (ref.)	0.94 (0.71 to 1.26)	0.87 (0.67 to 1.13)	0.93 (0.67 to 1.30)	0.52
<i>High TG</i>	<i>n = 569</i>	<i>n = 1,417</i>	<i>n = 4,444</i>	<i>n = 747</i>	
Incident cases n (%)	78 (13.7%)	159 (11.22%)	449 (10.10%)	95 (12.71%)	
Crude model	1 (ref.)	0.80 (0.60 to 1.06)	0.71 (0.55 to 0.92)*	0.92 (0.13 to 0.20)	0.62
Multivariable 1	1 (ref.)	0.83 (0.62 to 1.12)	0.72 (0.55 to 0.74)*	0.79 (0.56 to 1.10)	0.13
Multivariable 2	1 (ref.)	0.83 (0.61 to 1.11)	0.71 (0.54 to 0.93)*	0.79 (0.56 to 1.11)	0.13
Multivariable 3	1 (ref.)	0.83 (0.61 to 1.12)	0.71 (0.54 to 0.94)*	0.80 (0.56 to 1.12)	0.16

* $p < 0.05$; ** $p < 0.01$.

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

Multivariable 2: additionally adjusted for quartiles of body mass index (continuous), smoking status, hypertension at baseline, family history of cardiovascular disease, diabetes at baseline, physical activity during leisure time (continuous), adherence to the Mediterranean food pattern (continuous) and alcohol intake (continuous).

Multivariable 3: additionally adjusted for carbohydrate intake.

Table III. Multivariable-adjusted differences of blood concentrations of total cholesterol (TC), high-density lipoproteins (HDL-c) and triglycerides (TG) according to categories of egg consumption in the SUN cohort at 6 years of follow-up

	Categories of egg consumption				p for trend
	1	2	3	4	
	< 1 egg /week	1 egg/week	2-4 eggs/week	> 4 eggs/week	
<i>TC (mg/dl)</i>	<i>n = 363</i>	<i>n = 1,027</i>	<i>n = 3,256</i>	<i>n = 549</i>	
Crude model	0 (ref.)	+0.47 (-3.47 to +4.42)	+0.57 (-3.00 to +4.14)	-1.33 (-5.70 to +3.04)	0.48
Multivariable 1	0 (ref.)	+1.69 (-2.18 to +5.56)	+2.12 (-1.43 to +5.67)	+0.60 (-3.79 to +4.99)	0.96
Multivariable 2	0 (ref.)	+1.23 (-2.68 to +5.13)	+1.55 (-2.03 to +5.13)	-0.25 (-4.69 to +4.19)	0.78
<i>HDL-c (mg/dl)</i>	<i>n = 378</i>	<i>n = 968</i>	<i>n = 2,855</i>	<i>n = 477</i>	
Crude model	0 (ref.)	+0.85 (-1.77 to +3.47)	-0.00 (-2.37 to +2.36)	-3.43 (-6.40 to -0.45)*	0.004**
Multivariable 1	0 (ref.)	-0.05 (-2.56 to +2.46)	-0.49 (-2.78 to +1.80)	-2.03 (-4.93 to +0.88)	0.11
Multivariable 2	0 (ref.)	+0.43 (-2.11 to +2.98)	-0.16 (-2.49 to +2.17)	-2.02 (-4.98 to +0.95)	0.08
<i>TG (mg/dl)</i>	<i>n = 437</i>	<i>n = 1,124</i>	<i>n = 3,450</i>	<i>n = 747</i>	
Crude model	0 (ref.)	-7.60 (-12.66 to -2.53)**	-7.81 (-12.37 to -3.25)**	-6.77 (-12.49 to -1.05)*	0.17
Multivariable 1	0 (ref.)	-5.67 (-10.54 to -0.80)*	-5.69 (-10.13 to -1.25)*	-7.69 (-13.31 to -2.07)**	0.05
Multivariable 2	0 (ref.)	-6.31 (-11.14 to -1.48)*	-6.80 (-11.21 to -2.40)**	-7.91 (-13.51 to -2.30)**	0.04*
Multivariable 3	0 (ref.)	-5.99 (-10.83 to -1.16)*	-6.24 (-10.67 to -1.80)**	-7.24 (-12.87 to -1.60)*	0.08

* $p < 0.05$; ** $p < 0.01$.

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

Multivariable 2: additionally adjusted for quartiles of body mass index (continuous), smoking status, hypertension at baseline, family history of cardiovascular disease, physical activity during leisure time (continuous), adherence to the Mediterranean food pattern (continuous) and alcohol intake (continuous).

Multivariable 3: additionally adjusted for carbohydrate intake.

Table IV. Multivariable-adjusted differences of blood concentrations of total cholesterol (TC), high-density lipoproteins-(HDL) and triglycerides (TG) according to categories of egg consumption in the SUN cohort at 8 years of follow-up

	Categories of egg consumption				p for trend
	1	2	3	4	
	< 1 egg/week	1 to 2 eggs/week	2-4 eggs/week	> 4 eggs/week	
<i>TC (mg/dl)</i>	<i>n</i> = 315	<i>n</i> = 803	<i>n</i> = 2,710	<i>n</i> = 481	
Crude model	0 (ref.)	-2.32 (-6.71 to -2.06)	-1.64 (-5.56 to 2.29)	-0.05 (-4.83 to +4.73)	0.53
Multivariable 1	0 (ref.)	-1.39 (-5.70 to +2.91)	-0.30 (-4.20 to +3.60)	+1.17 (-3.63 to +5.97)	0.28
Multivariable 2	0 (ref.)	-1.85 (-6.21 to +2.51)	-0.71 (-4.67 to +3.24)	+1.00 (-3.87 to +5.87)	0.27
<i>HDL-c (mg/dl)</i>	<i>n</i> = 333	<i>n</i> = 791	<i>n</i> = 2,468	<i>n</i> = 419	
Crude model	0 (ref.)	-0.16 (-3.17 to +2.86)	-0.42 (-3.11 to +2.28)	-4.56 (-7.95 to -1.17)*	0.01*
Multivariable 1	0 (ref.)	-0.86 (-3.79 to +2.07)	-0.78 (-3.44 to +1.87)	-3.28 (-6.64 to +0.09)	0.09
Multivariable 2	0 (ref.)	-0.52 (-3.48 to +2.43)	-0.98 (-3.66 to +1.71)	-4.01 (-7.42 to -0.61)*	0.02*
<i>TG (mg/dl)</i>	<i>n</i> = 378	<i>n</i> = 906	<i>n</i> = 2,919	<i>n</i> = 498	
Crude model	0 (ref.)	-6.06 (-11.47 to -0.64)*	-6.90 (-11.73 to -2.06)**	-5.20 (-8.63 to +3.43)	0.78
Multivariable 1	0 (ref.)	-5.30 (-10.53 to -0.07)*	-6.15 (-10.88 to -1.42)*	-5.20 (-11.14 to +0.75)	0.22
Multivariable 2	0 (ref.)	-4.95 (-10.15 to +0.25)	-5.81 (-10.51 to -1.10)*	-4.01 (-9.94 to +1.92)	0.38
Multivariable 3	0 (ref.)	-4.92 (-10.12 to +0.29)	-5.74 (-10.48 to -1.01)*	-3.94 (-9.90 to +2.03)	0.41

p* < 0.05; *p* < 0.01.

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

Multivariable 2: additionally adjusted for quartiles of body mass index (continuous), smoking status, hypertension at baseline, family history of cardiovascular disease, physical activity during leisure time (continuous), adherence to the Mediterranean food pattern (continuous) and alcohol intake (continuous).

Multivariable 3: additionally adjusted for carbohydrate intake.

When we repeated this assessment using HDL-c values after 8-years of follow-up we did find an association between egg consumption and lower HDL-c levels (*p* for trend = 0.02) with a fully adjusted difference of -4.01 (-7.42 to -0.61) mg/dl in HDL-c between extreme categories of egg consumption (> 4 vs. < 1 egg/week). Results for blood levels of triglycerides showed lower means of triglycerides in each of the three upper categories of egg consumption than in the lowest category (< 1 egg/week). Results were statistically significant for all comparisons after 6-year follow-up and for many of them after 8-year follow-up. However, the linear trend test was never significant in the follow-up after 8-years (Table IV), it lost its statistical significance in the fully-adjusted model after 6 years (Table III) and the point estimates suggested a plateau effect instead of a monotonically decreasing trend. As sensitivity analyses we fitted logistic regression and multiple linear regression models after adjusting for BMI as a continuous variable instead of quartiles of BMI and we observed similar results (data not shown). We did not observe any significant interaction between egg consumption and adherence to the Mediterranean dietary pattern (cut-off ≥ 6 vs. < 6 in the 9-item Trichopoulou score) (31).

DISCUSSION

This longitudinal study did not identify any clear harmful association between egg consumption and the incidence of hyper-

cholesterolemia or hypertriglyceridemia in a prospective cohort of highly educated Mediterranean subjects. We did observe an inverse association between egg consumption and mean serum HDL-c levels with a decreasing linear trend that was statistically significant only after 8 years of follow-up. Lower average concentrations of HDL-c were observed in subjects who consumed more than 4 eggs/week after adjusting for a wide array of potential confounders. However, the size of the difference was small and probably it was not clinically relevant, because we did not observe any increased risk of a new diagnosis of low HDL levels (considered as a clinically relevant dichotomous outcome). On the other hand, we found a reduction in the incidence of medically-diagnosed hypertriglyceridemia and lower average levels of triglycerides in subjects who had higher egg consumption. Due to the fact that egg is the major source of dietary cholesterol, metabolic studies have focused on the effect of dietary cholesterol contained in eggs on serum lipids but their influences on plasma concentrations of serum cholesterol the effect is quite small (32,33). Thus, it seems more appropriate to recommend healthy dietary habits and active lifestyle instead of recommendations focused on the restriction of dietary cholesterol from eggs. In this same cohort we found no association between egg consumption and the incidence of CVD among 14,185 university graduates (34). Furthermore, in a prospective cohort study of 21,327 participants from the Physicians' Health Study, the consumption of ≥ 6 eggs/week had no major

effect on the risk of CVD and mortality and the consumption of ≥ 7 eggs/week was associated with a modestly greater risk of total mortality (35-37). Other studies with experimental designs (4) reported that daily whole egg consumption during moderate carbohydrate restriction diets were associated with greater increases in plasma HDL-c and improvements in HDL-c profiles in patients with the MetS (38-40). Therefore, our unexpected result of an inverse association with HDL-c in some analyses (only present after 8 years), should be viewed with caution, because it is not consistent with previously reported results and with our analyses after 6 years. Approximately one third of individuals tend to have a high plasma response to dietary cholesterol (hyper-responders). The increases in LDL-c and HDL-c due to increased egg consumption in hyper-responders are not likely to be related to an increased number of LDL or HDL-c particles but, to an increase in the less atherogenic lipoprotein sub-fractions. These data suggested that additional dietary cholesterol did not increase the risk of developing an atherogenic lipoprotein profile in healthy men, regardless of their response classification.

The most apparent potential limitation of our study is the self-report nature of lipid levels, but these self-reported values were previously validated in specific studies conducted in sub-samples of the SUN cohort (18,19). A second potential concern may be related to the generalizability of our findings which are based on a young cohort of university graduates that is a non-representative sample of the general population. On the other hand, their high educational level ensures a more accurate response. In addition, egg consumption might be underestimated because FFQs inquired about units of egg consumed and the amount of eggs contained in other products (pastries or sauces), were not considered. In addition, we only assessed egg consumption at baseline. However, several important strengths are the prospective design and large sample size of our study, our long-term follow-up, a high retention rate (81.5%), the ability to control for a wide array of potential confounders, the robustness of our results in sensitivity analyses and the existence of published validation studies for self-reported measurements, including dietary assessment (18,21,22,25,27,28).

Our data do not support that higher egg consumption is associated with abnormal blood levels of total cholesterol or triglycerides; however, an inverse association with HDL-c as a quantitative variable was found in only one of our assessments and future studies are warranted to confirm this result. Results from the SUN cohort do not provide any evidence to recommend a reduction in egg consumption in healthy subjects as a means to reduce lipid levels.

ACKNOWLEDGEMENTS

The authors thank the participants of the SUN study for their continued cooperation and participation and all members of the SUN Study Group. We are also grateful to the members of the Department of Nutrition of the Harvard School of Public Health (Willett WC, Hu FB, and Ascherio A) who helped us to design the SUN study. The SUN Project has received funding from the Spanish Government-Instituto de Salud Carlos III, and the Euro-

pean Regional Development Fund (FEDER) (RD 06/0045, CIBER OBN, Grants PI10/02658, PI10/02293, PI13/00615, PI14/01668, PI14/01798, PI17/01795, and G03/140), the Navarra Regional Government (45/2011, 122/2014), and the University of Navarra.

REFERENCES

- Spence JD, Jenkins DJA, Davignon J. Dietary cholesterol and egg yolks: not for patients at risk of vascular disease. *Can J Cardiol* 2010;26:336-9.
- Berger S, Raman G, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis. *Am J Clin Nutr* 2015;102:276-94.
- Waters D, Clark RM, Greene CM, Contois JH, Fernandez ML. Change in plasma lutein after egg consumption is positively associated with plasma cholesterol and lipoprotein size but negatively correlated with body size in postmenopausal women. *J Nutr* 2007;137:959-63.
- Andersen CJ, Blesso CN, Lee J, Barona J, Shah D, Thomas MJ, et al. Egg consumption modulates HDL lipid composition and increases the cholesterol-accepting capacity of serum in metabolic syndrome. *Lipids* 2013;48:557-67.
- Greene CM, Waters D, Clark RM, Contois JH, Fernandez ML. Plasma LDL and HDL characteristics and carotenoid content are positively influenced by egg consumption in an elderly population. *Nutr Metab* 2006;3:6.
- Shin JY, Xun P, Nakamura Y, He K. Egg consumption in relation to risk of cardiovascular disease and diabetes: a systematic review and meta-analysis. *Am J Clin Nutr* 2013;98:146-59.
- Rong Y, Chen L, Zhu T, Song Y, Yu M, Shan Z, et al. Egg consumption and risk of coronary heart disease and stroke: dose-response meta-analysis of prospective cohort studies. *BMJ* 2013;346:e8539.
- Fuertes Garcia A. Consumo de huevos y enfermedad cardiovascular. *Nutr Hosp* 2016;33:41-3.
- Scientific Report of the 2015 Dietary Guidelines Advisory Committee (DGAC) (2015) Evidence Basis and Key Recommendations. Available at: <http://health.gov/dietaryguidelines/2015-scientific-report/06-chapter-1/d1-2.asp> (accessed March 2016).
- Levin S, Wells C, Barnard N. Dietary Cholesterol and Blood Cholesterol Concentrations. *Jama* 2015;314:2083-4.
- Schersten T, Rosch PJ, Arfors KE, Sundberg R. The cholesterol hypothesis: time for the obituary? *Scand Cardiovasc J* 2011;45:322-3.
- KA SW, Krause AJ, Shearer S, Devries S. The 2015 Dietary Guidelines Advisory Committee Report Concerning Dietary Cholesterol. *Am J Cardiol* 2015;116:1479-80.
- Komaroff AL. By the way, doctor. Ever since the 1960s, I haven't eaten more than one egg a week because egg yolks have a lot of cholesterol. Now my wife says she heard on the news that it's okay to eat eggs every day. What do you think? *Harv Health Lett* 2005;30:8.
- Schonfeld G, Patsch W, Rudel LL, Nelson C, Epstein M, Olson RE. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J Clin Invest* 1982;69:1072-80.
- Anand SS, Hawkes C, de Souza RJ, Mente A, Dehghan M, Nugent R, et al. Food Consumption and its Impact on Cardiovascular Disease: Importance of Solutions Focused on the Globalized Food System: A Report From the Workshop Convened by the World Heart Federation. *J Am Coll Cardiol* 2015;66:1590-614.
- Martinez-Gonzalez MA, Sanchez-Villegas A, Irala J De, Marti A, Martinez JA. Mediterranean diet and stroke: objectives and design of the SUN project. *Seguimiento Universidad de Navarra. Nutr Neurosci* 2002;5:65-73.
- Martinez-Gonzalez MA. The SUN cohort study (Seguimiento University of Navarra). *Public Health Nutr* 2006;9:127-31.
- Barrio-Lopez MT, Bes-Rastrollo M, Beunza JJ, Fernandez-Montero A, Garcia-Lopez M, Martinez-Gonzalez MA. Validation of metabolic syndrome using medical records in the SUN cohort. *BMC Public Health* 2011;11:867.
- Fernandez-Montero A, Beunza JJ, Bes-Rastrollo M, Barrio MT, de la Fuente-Arillaga C, Moreno-Galarraga L, et al. Validity of self-reported metabolic syndrome components in a cohort study. *Gac Sanit* 2011;25:303-7.
- Willett WC. *Issues in Analysis and Presentation of Dietary Data*. In: 2nd edition. New York: Oxford University Press; 1998. pp. 322.
- Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol* 1993;22:512-9.

22. de la Fuente-Arrillaga C, Ruiz ZV, Bes-Rastrollo M, Sampson L, Martínez-González MA. Reproducibility of an FFQ validated in Spain. *Public Health Nutr* 2010;13:1364-72.
23. Moreiras O, Carbajal A, Cabrera L, Cuadrado C. *Tabla de composición de alimentos*. 16th ed. Madrid: Piramide; 2013.
24. Mataix J. *Tabla de composición de alimentos*. 5th ed. Granada: Universidad de Granada; 2009.
25. Martínez-González MA, Lopez-Fontana C, Varo JJ, Sanchez-Villegas A, Martínez JA. Validation of the Spanish version of the physical activity questionnaire used in the Nurses' Health Study and the Health Professionals' Follow-up Study. *Public Health Nutr* 2005;8:920-7.
26. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000;32:498-504.
27. Bes-Rastrollo M, Pérez JR, Sánchez-Villegas A, Alonso A, Martínez-González MA. Validation of self reported weight and body mass index in a cohort of university graduates in Spain. *Rev Esp Obes* 2005;3:352-8.
28. Alonso A, Beunza JJ, Delgado-Rodríguez M, Martínez-González MA. Validation of self reported diagnosis of hypertension in a cohort of university graduates in Spain. *BMC Public Health* 2005;5:94.
29. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;348:2599-608.
30. National Cholesterol Education Program. High Blood Cholesterol ATP III Guidelines At-A-Glance Quick-Desk-Reference. U.S. Department of Health and Human Services Public Health Service National Institutes of Health National Heart, Lung, and Blood Institute. Available at: <https://www.nhlbi.nih.gov/files/docs/guidelines/atglance.pdf>
31. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean Diet and Survival in a Greek Population. *N Engl J Med* 2003;348:2599-608.
32. Kanter MM, Kris-Etherton PM, Fernandez ML, Vickers KC, Katz DL. Exploring the factors that affect blood cholesterol and heart disease risk: is dietary cholesterol as bad for you as history leads us to believe? *Adv Nutr* 2012;3:711-7.
33. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am J Clin Nutr* 1997;65:1747-64.
34. Zazpe I, Beunza JJ, Bes-Rastrollo M, Warnberg J, de la Fuente-Arrillaga C, Benito S, et al. Egg consumption and risk of cardiovascular disease in the SUN Project. *Eur J Clin Nutr* 2011;65:676-82.
35. Djousse L, Gaziano JM. Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. *Am J Clin Nutr* 2008;87:964-9.
36. Alexander DD, Miller PE, Vargas AJ, Weed DL, Cohen SS. Meta-analysis of Egg Consumption and Risk of Coronary Heart Disease and Stroke. *J Am Coll Nutr* 2016;35(8):704-16.
37. Díez-Espino J, Basterra-Gortari FJ, Salas-Salvado J, Buil-Cosiales P, Corella D, Schröder H, et al. Egg consumption and cardiovascular disease according to diabetic status: The PREDIMED study. *Clin Nutr Clin Nutr* 2017;36(4):1015-21. DOI: 10.1016/j.clnu.2016.06.009
38. Blesso CN, Andersen CJ, Bolling BW, Fernandez ML. Egg intake improves carotenoid status by increasing plasma HDL cholesterol in adults with metabolic syndrome. *Food Funct* 2013;4:213-21.
39. Klangjareonchai T, Putadechakum S, Sritara P, Roongpisuthipong C. The Effect of Egg Consumption in Hyperlipidemic Subjects during Treatment with Lipid-Lowering Drugs. *J Lipids* 2012;2012:672720.
40. Woo HW, Choi BY, Kim MK. Cross-Sectional and Longitudinal Associations between Egg Consumption and Metabolic Syndrome in Adults \geq 40 Years Old: The Yangpyeong Cohort of the Korean Genome and Epidemiology Study (KoGES_Yangpyeong). *PLoS One* 2016;11:e0147729.