



*Original Article*

## The effect of ingestion of egg and low density lipoprotein (LDL) oxidation on serum lipid profiles in hypercholesterolemic women

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### Abstract

Egg is a major source of dietary cholesterol. The serum lipid response to egg shows marked individual variation, being partly genetically determined, and influence by ethnic groups and the overall diet response. In the present investigation, we investigated the effect of ingestion of egg and low density lipoprotein (LDL) oxidation on serum lipid profile in hypercholesterolemic women. Forty hypercholesterolemic women volunteers on a cholesterol-lowering diet (CLD) divided into 2 groups in a randomized controlled cross-over study of one egg per day (CLD + 1 egg) for 4-week and three eggs per day (CLD + 3 eggs) for 4-week, separated by 4-week period egg-free. The body weight, blood pressure, serum lipid profiles and LDL oxidation were measured at 4-week intervals. Cholesterol-lowering diet was applied throughout the study by a dietitian using a food exchange program and 3-day dietary recall every 4 weeks. Compared to the values obtained at baseline, the mean serum total cholesterol and LDL cholesterol of CLD + 3 eggs was not significantly different from baseline whereas of those of 4-week of egg-free period and CLD + 1 egg were significantly decreased (238.3±2.9 mg/dL and 228.3±4.7 mg/dL) compared to the baseline (252.2±5.9 mg/dL) as was LDL cholesterol (161.2±3.0 mg/dL and 155.7±4.8 mg/dL) compared to the baseline (177.5±6.0 mg/dL) ( $p<0.05$ ). The study showed there were no significantly difference the body weight, blood pressure, HDL cholesterol, triglycerides or LDL oxidation during the study. However, serum total cholesterol and LDL cholesterol of 1 or 3 eggs per day after 4-week of egg consumption was not significantly higher than the egg-free period. The study suggests that in hypercholesterolemic women who are on cholesterol-lowering diet, consuming one or three eggs per day did not raise serum cholesterol or LDL cholesterol levels at 4 weeks or result in any change in LDL oxidation.

**Keywords:** egg consumption, serum lipids, hypercholesterolemic women, cholesterol-lowering diet, LDL oxidation

### 1. Introduction

Coronary heart disease (CHD) is an important leading cause of death in the modern world, even in the developing countries such as Thailand. The prevalence of CHD has been increasing in Thailand. Elevated LDL cholesterol has been

identified as a major risk factor for the development and progression of CHD. It has emerged as one of the most common risk factors of CHD, and has become a significant public health problem (Sritara *et al.*, 2003). To avoid elevating blood cholesterol and to reduce CHD risk, it has been generally advised since 1970 to consume no more than 300 mg/d of cholesterol and limit consumption of eggs to no more than 3 to 4 whole eggs per week (Kritchevsky, 2004). Egg is a major source of dietary cholesterol with an average of 200 mg cholesterol in one egg. There is a large variability in indivi-

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dual response to egg in terms of cholesterol level and cardiovascular events (Chakrabarty *et al.*, 2002; Pyorala, 1987; Ballesteros *et al.*, 2004). Some studies have shown positive associations between dietary and serum cholesterol (Mattson *et al.*, 1972; Keys, 1984; Weggemans *et al.*, 2001; Sacks *et al.*, 1984; Hu *et al.*, 1999), others did not find any effect (Chakrabarty *et al.*, 2002; Pyorala, 1987). In 1999, a large prospective cohort study of egg consumption and risk of cardiovascular disease in men and women showed no significant relationship between egg consumption and CHD or stroke in either men or women (Hu *et al.*, 1999). There are some papers showing an effect of sex on serum lipid profiles due to the hormonal changes. One study has reported a dose-response relationship between egg consumption and total cholesterol level and all-cause mortality in women but not in men (Nakamura *et al.*, 2004).

Eggs are not the only major sources of dietary cholesterol (200 mg/egg) but also provide high-quality, bioavailable protein with little total fat. Compared to other animal protein sources, eggs contain proportionately less saturated fat. Eggs are an excellent natural source of folate, riboflavin, selenium, choline, vitamin B12, and fat-soluble vitamins A, D, E, and K (Song and Kerver, 2000) that could reduce the risk of coronary artery disease (CAD). In addition, consumption of eggs instead of carbohydrate-rich foods may raise high-density lipoprotein (HDL) cholesterol levels (Schnohr *et al.*, 1994; Mayurasakorn *et al.*, 2008) and decrease blood glycemic and insulinemic responses (Pelletier *et al.*, 1996). Eggs are an inexpensive and low-calorie source of high-quality protein and other nutrients. Thus, it is important to look at eggs as more than a cholesterol-delivery system. In Japan and Hongkong the population has egg consumption of around 390 and 230 capita per year. In Thailand, a reduction in consumption of eggs, a concentrated source of cholesterol (one yolk provides ~215 mg of cholesterol), had been widely recommended in an effort to lower blood cholesterol and reduce the risk of heart disease (Report of Inter-Society Commission for Heart Disease Resources: Prevention of Cardiovascular Disease, 1970). However, more recent American Heart Association (AHA) guidelines no longer advise for or against egg or egg yolk consumption (Krauss *et al.*, 2000) but Thai people still consume eggs at only one-third of the rate among the Japanese population. To the best of our knowledge, no study has ever compared the effects of egg consumption in hyperlipidemic female. Therefore, we performed a randomized cross-over trial to assess the effects of egg consumption on lipid panel and LDL oxidation in hyperlipidemic women.

## 2. Materials and Methods

### 2.1 Subjects

Forty female volunteers aged 30 to 60 years with serum cholesterol level  $\geq 200$  mg/ml were recruited. Subjects were

excluded if they had egg allergies, hypothyroidism, documented heart disease, diabetes mellitus, liver or kidney problems or were using hormonal contraceptives or were continuously receiving lipid-lowering medication. They recorded dietary intake for 3 days and were taught by a dietitian to control body weight with a cholesterol-lowering diet (CLD) throughout the study.

After a 4-week CLD which was a run-in period, they were randomly divided into 2 groups ( $n=20$  each) for a randomized crossover trial of two 4-week interventions, one egg per day (CLD + 1 egg) for 4 weeks and three eggs per day (CLD + 3 eggs) for 4 weeks, separated by 4-week egg-free period (washout period). The body weight, body mass index, blood pressure, serum lipid profiles and 3-day 24-hour dietary recall were recorded every 4 weeks. The Biochemical tests of blood sugar, renal and liver function tests and hematological parameters were measured before and after the study.

The study was reviewed and approved by the Committee on Human Research of Ramathibodi Hospital, Mahidol University. The participants were informed in detail about the study and informed consents was obtained from all of them.

### 2.2 Diet designs

The CLD was composed of 50 to 60% of calories from carbohydrates, 15% from protein, and 25 to 30% from total fat, which contained less than 10% in saturated fat, more than 10% in monounsaturated fat and 10% in polyunsaturated fat. Twenty-four hour dietary recall was recorded by dietitians to determine the average daily intake of energy and certain nutrients (carbohydrate, total protein, fat, dietary cholesterol and calories) and then the Thai food exchange menu was recommended for each subject. Lunch meal with egg(s) supplement was provided by the research kitchen everyday. Dietary records were recall every 4 weeks during the study.

### 2.3 Lipid profile

Serum was drawn for lipid assessment after 12 hours overnight fast. The lipid profile was determined as follows: total cholesterol (TC) was determined by enzymatic colorimetric method (Warnick *et al.*, 1989), triglycerides (TG), was determined by enzymatic hydrolysis with subsequent determination of the liberated glycerol by Boeringer Mannheim GPO-PAP Kit (Sampson *et al.*, 1994) and high-density lipoprotein (HDL) was determined by Lopes-Virella method (Lopes-Virella *et al.*, 1977). Low-density-lipoprotein (LDL) was obtained by calculation:  $LDL = TC - (TG/5 + HDL)$  (Friedewald *et al.*, 1972).

### 2.4 Body weight

Body weight was measured using a beam balance-type medical scale with light clothing and without shoes at the begin-

ning and every 4-week. and was taken to the nearest 0.1 kg. Body mass index (BMI) was calculated from body weight and height as  $BW(kg)/Height^2(m^2)$

### 2.5 Blood pressure

Blood pressure was determined with the use of the Datascope Accutorr Plus automatic digital blood pressure device (Datascope Corp, Mahwah, NJ) with the participant supine after a 5-min period of rest. Both systolic and diastolic pressures were calculated as the mean of 2 readings 5 minutes apart. All measurements were obtained by one investigator.

### 2.6 Lipoprotein fractions

Lipoprotein fractions were separated from plasma by ultra-centrifuge at 45,000 rpm at 4°C for 8 hours. LDL was isolated from freshly prepared plasma by sequential ultra-centrifugation technique (Harvel *et al.*, 1955).

### 2.7 LDL oxidation

The methods for LDL oxidation by copper followed those of Lowry and colleagues and Esterbauer and colleagues (Esterbauer *et al.*, 1989; Lowry *et al.*, 1951)

### 2.8 Statistical analysis

Statistical analysis was conducted using SPSS software (Version 11.5 for Windows). A two-tailed  $p$ -value of  $\leq 0.05$  was considered statistically significant. Repeated measures ANOVA was used to test serum lipid profiles and

LDL oxidation and the Bonferroni multiple comparison method applied. Unpaired t-test was used to test the difference in general characteristics between the 2 groups. Sample size was predicated on 80% power to detect a minimal difference of 3.5% change in flow-mediated dilation (FMD) between the dietary regimens at 4 weeks. A two-tailed alpha level of 0.05 was set with an allowance for 10% attrition and noncompliance.

## 3. Results

Forty hyperlipidemic females participated in this study. Demographic and baseline characteristics are shown in Table 1. Eight participants dropped out of the study. Five participant dropped out because the participant was unwilling to consume 3 eggs daily for 4 weeks, another 2 dropped out because of inconvenience in taking the free lunch egg meal from the hospital, and one dropped out because she was diagnosed as having carcinoma of the breast. Baseline TC, LDL, HDL and TG were  $252.2 \pm 5.9$ ,  $177.5 \pm 6.0$ ,  $51.8 \pm 1.4$  and  $110.8 \pm 7.9$  mg/dL, respectively.

After the periods of CLD for 4 weeks and of CLD + 1 egg for 4 weeks the TC was significantly lower as compared to baseline ( $p < 0.05$ ) as was the level of LDL ( $p = 0.05$ ). However, daily CLD + 3 eggs for 4 weeks did not significantly lower TC as compared to the baseline (Table 2). Daily consumption of either 1 egg or 3 eggs per day for 4 weeks did not significantly raise TC or lag time of LDL oxidation as compared to the egg-free regimens for 4 weeks (Table 3).

The study also showed no significant change of body weight, body mass index, HDL, TG or lag time of LDL oxidation throughout the study. The biochemical tests of blood

Table 1. Baseline characteristics of the subjects

Parameter	Group		p-value
	Group 1	Group 2	
Sex (female)	16	16	
Age (year)	40.1 $\pm$ 10.2	38.2 $\pm$ 9.5	0.581
Body weight (kilogram)	55.6 $\pm$ 8.5	55.4 $\pm$ 8.7	0.961
Height (meter)	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1	0.654
BMI (kg/m <sup>2</sup> )	22.7 $\pm$ 4.3	22.3 $\pm$ 3.4	0.733
SBP (mm Hg)	110.0 $\pm$ 9.6	112.1 $\pm$ 18.9	0.654
DBP (mm Hg)	73.5 $\pm$ 5.3	71.3 $\pm$ 7.2	0.691
Pulse rate (beat/min)	73.4 $\pm$ 7.0	72.5 $\pm$ 4.9	0.684
TC (mg/dL)	251.3 $\pm$ 24.3	253.0 $\pm$ 40.9	0.888
LDL (mg/dL)	115.6 $\pm$ 48.2	106.13 $\pm$ 42.5	0.953
HDL (mg/dL)	51.0 $\pm$ 8.8	52.6 $\pm$ 6.7	0.561
TG (mg/dL)	177.2 $\pm$ 23.7	177.90 $\pm$ 42.1	0.561

BMI = Body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol, LDL = low density lipoprotein, HDL = high density lipoprotein, TG = triglyceride

Table 2. Mean and standard error of mean (SEM) of serum lipid profiles during the study

Parameter	Baseline diet	CLD	CLD and 1 egg per day	CLD and 3 egg per day
TC (mg/dL)	252.2±5.9 <sup>a</sup>	238.3±2.9 <sup>b</sup>	228.3±4.7 <sup>b</sup>	239.6±5.4 <sup>ab</sup>
LDL (mg/dL)	177.5±6.0 <sup>a</sup>	161.2±3.0 <sup>b</sup>	155.7±4.8 <sup>b</sup>	166.7±5.0 <sup>ab</sup>
HDL (mg/dL)	51.8±1.4	53.8±1.4	51.28±1.7	50.6±1.4
TG (mg/dL)	110.8±7.9	116.3±6.7	106.4±6.4	111.8±7.9

<sup>a, b</sup> Values not having a superscript in column within the same row are significantly different (p<0.05)

Table 3. Mean and standard error of mean (SEM) of lag time of LDL oxidation during the study

Parameter	CLD	CLD and 1 egg per day	CLD and 3 eggs per day
Lag time (min)	62.09±1.71	60.38±2.16	62.22±1.79

sugar, renal and liver function tests and hematological test were not significantly different between before and after the study.

**4. Discussion**

Our findings showed that short-term consumption of eggs daily did not unfavorably influence serum cholesterol or other measures of the lipid profile in hyperdemic women a population not previously examined; the study indicated that the addition of either one egg or three eggs per day with a cholesterol-lowering diet (CLD) in borderline high total cholesterolemic women does not result in any increase of serum TC or LDL as compared to CLD which is egg-free for 4 weeks. This phenomenon may due to the maintainance CLD throughout the study. As in our study all the borderline high total cholesterolemic women were on diet control which limited dietary fat and cholesterol and also concentrated to variation of types of fatty acid. We already known that the serum TC and LDL does not depend only on the amount of cholesterol intake in the diet but also on the kind of fat in the diet as defined in Keys' formula (Keys, 1984)

$$C = 1.35(2S - P) + 1.5z$$

Whereas

- C = change in serum TC (mg/dl)
- S = change in percent energy intake from saturated fatty acids
- P = change in percent energy from polyunsaturated fatty acids
- z = difference in square root of baseline versus treatment cholesterol intakes (mg/1000 kcal)

Our work repudiates the hypothesis that increased egg consumption leads to increased serum cholesterol con-

centrations. This finding is consistent with the prior study of egg ingestion in healthy young Indians (Chakrabarty *et al.*, 2004). The researchers who reported eating 4 eggs per week had a significantly lower mean serum cholesterol concentration than those who reported eating 1 egg per week (193 mg/dL vs. 197 mg/dL, *p* < 0.01). More frequent egg consumption was negatively associated with serum cholesterol concentration ( $\beta = -6.45, p < 0.01$ ) (Song and Kerver, 2000). Previous studies have shown weak positive associations between intake of dietary cholesterol and serum cholesterol, while others failed to find any association (McNamara, 2009). While rich in cholesterol, eggs are also nutritious. Data from NHANES III reveal that egg consumption is an important nutritional contribution to the average American diet, providing a relatively inexpensive source of amino acids and essential fatty acids (Song and Kerver, 2000). Lag time of LDL oxidation was consistent with the cross-over study of Greene *et al.* (2005) that compared consumption of 3 eggs per day and egg substitute for 30 days, with a washout period 3 weeks. There were no changes in the lag time for 42 normal subjects who mean no association of adverse effect of higher eggs consumption on lipid profiles and LDL oxidation. Thus, in pregnant woman, elderly or patients with malnutrition, recommendation of egg supplement for increasing protein consumption may be suitable because of its enrich the nutrients, inexpensive cost and not increase serum cholesterol level at lease in 1 month.

Although our results suggest that higher egg consumption is not associated with higher serum cholesterol, this study should not be used as a basis for recommending higher egg consumption for regulation of serum cholesterol. Furthermore, the duration of egg consumption during this study limits the ability to predict the long-term effects. Variables potentially confounding the correlation between nutrient intakes and serum total cholesterol level include physical activity, dietary intake, and genetic factors (Morgan *et al.*, 1986; Njike *et al.*, 2010).

## 5. Conclusions

The borderline high total cholesterolemic women who consumed either 1 or 3 eggs per day did not show any increase of total cholesterol, LDL cholesterol level or LDL oxidation while on cholesterol-lowering diet.

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