Note

The Effect of the Consumption of Egg on Serum Lipids and Antioxidant Status in Healthy Subjects

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Summary
The egg is a nutrient-dense food and contains a number of antioxidants. The consumption of eggs has been considered to improve the balance of diets, although its impact on serum cholesterol levels has been a matter of concern in many countries. Here, we conducted a pilot study to investigate whether daily additional consumption of an egg might affect serum lipid profiles and also antioxidant status in healthy subjects. Fourteen male subjects were provided with breakfasts including a boiled egg for 4 wk. At the end of intervention, serum concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were unchanged, despite the significant increase in the intake of dietary cholesterol. In contrast, a significant increase in high-density lipoprotein cholesterol (HDL-C) and a reduction of the LDL-C/HDL-C ratio were observed. Interestingly, the malondialdehyde modified-LDL (MDA-LDL)/LDL-C ratio and the oxidizability of LDL were significantly reduced. Serum total antioxidant capacity value after the intervention period was higher than at baseline. These data indicate that consuming one egg per day for 4 wk as breakfast in addition to a normal diet does not affect serum lipids, and suggests that it may improve serum antioxidant status in healthy males.

Key Words egg, dietary cholesterol, serum lipids, antioxidants, LDL oxidation

Eggs are a major source of dietary cholesterol and many clinical trials have investigated the relationship between egg consumption and lipid profiles. In such studies, however, greater doses of cholesterol than the average Japanese intake have typically been employed as intervention. The mean cholesterol intakes of Japanese adults are 338 mg/d for men and 279 mg/d for women (1), while the doses used in many trials ranged from 500 to 1,400 mg/d (2). Thus, further trials with moderate cholesterol intakes are indispensable to better understand the possible effects of typical cholesterol intakes in healthy populations.

Beside the possible adverse aspect due to their cholesterol content, eggs are an important source of high-quality protein, and eggs are rich in other key nutrients, except for vitamin C and dietary fiber. Eggs are also a rich source of lutein and zeaxanthin, which are powerful antioxidative carotenoids. Carotenoids and dietary cholesterol share intestinal absorptive pathways, and lipoproteins are the major carriers of carotenoids in circulation (3). Lutein and zeaxanthin in eggs are known to be highly bioavailable as compared with those in other sources such as spinach (4). A 5-wk intervention study demonstrated that consuming only one egg/d in elderly subjects significantly increased both serum lutein and zeaxanthin concentrations without elevating serum total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C) concentrations (5). However, the effect of daily egg consumption on antioxidant status in serum and lipoproteins in humans has not been well elucidated.

Here, we conducted a pilot study to investigate whether additional daily consumption of one egg for 4 wk affected serum lipid profiles or antioxidant status in healthy Japanese males.

Materials and Methods
Subjects. Fifteen healthy male volunteers were recruited from university communities by public offering through a recruitment poster with the following exclusion criteria: (1) allergy to eggs; (2) use of lipid-lowering medication. The study was approved by the Ethics Committee of Ochanomizu University, conformed to the Declaration of Helsinki, and was registered at UMIN-CTR as UMIN000011931. All subjects gave their written informed consent to participate in the study.

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Experimental design and diets. A single-arm, open-label, single-blind, randomized controlled clinical study was performed for 4 wk. An egg was provided as part of a breakfast that consisted of one boiled egg, one piece of white toast with margarine/jam, vegetable salad, low-sugar yogurt, fruit juice and black tea. The average cholesterol content in a single boiled egg (Kewpie Corporation, Tokyo, Japan) was 175 mg. Amounts of the lutein, zeaxanthin and α-tocopherol content were 0.181, 0.136 and 0.545 mg/egg, respectively. Total energy was 546 kcal (2,284 kJ), and the macronutrient composition of this breakfast was approximately 60.0% energy from carbohydrates, 14.8% energy from protein, and 25.2% energy from fat. The subjects were instructed to maintain their regular dietary habits (including alcohol and supplement consumption) except for breakfast and to record and take pictures of the contents of daily meals, snacks, and beverages during the test period. Consumption of neither eggs nor egg-containing foods other than the provided egg was restricted during the intervention period.

Dietary assessments. Basal dietary analysis was conducted for 1 wk before the test period. The diet diary was collected every weekday and daily intake of each component was calculated by an experienced dietitian on the basis of the 2010 of the Standard Tables of Food Composition in Japan (6).

Study visits and blood samples. Subjects attended two visits to the laboratory, in the morning between 7:00 and 8:30 am, 4 wk apart. In the 12 h before the blood samplings, participants were not permitted to eat or drink except for water and to smoke. Weight, abdominal circumference and blood pressure were measured. Blood samples were drawn from an antecubital vein and collected into tubes containing ethylenediamine-tetraacetic acid (EDTA) or clot accelerant for the immediate separation of plasma and serum.

Biochemical analyses. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride, phospholipids, and nonesterified fatty acid (NEFA) were measured by commercially available enzymatic assays using an autoanalyzer. LDL-C was calculated using the Friedewald formula. Serum concentrations of apolipoprotein (Apo)-A1 and B were measured using turbidimetric immunoassays (Sekisui Medical, Tokyo, Japan). Malondialdehyde modified-LDL (MDA-LDL) concentration was measured by enzyme-linked immunosorbent assay. Serum total antioxidant capacity was determined by 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay (Cayman Chemical, Ann Arbor, MI), according to the manufacturer’s instructions. Serum lutein + zeaxanthin and vitamin C concentrations were measured by high performance liquid chromatography, and vitamin E concentration was measured by the fluorometric method.

Table 1. Daily dietary intakes of subjects at baseline and during the 4-wk intervention.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>During test period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>2,151±474 kcal</td>
<td>2,329±463</td>
</tr>
<tr>
<td>Protein</td>
<td>80.1±12.7 g</td>
<td>87.8±17.2*</td>
</tr>
<tr>
<td>Fat</td>
<td>69.8±15.4 g</td>
<td>76.3±15.1</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>20.4±5.8 g</td>
<td>21.1±4.3</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>26.1±4.6 g</td>
<td>29.4±5.8*</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>13.5±3.2 g</td>
<td>15.2±3.5*</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.69±0.20 (ratio)</td>
<td>0.72±0.09</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>379±120 mg</td>
<td>533±102***</td>
</tr>
<tr>
<td>Cholesterol from eggs</td>
<td>160±87 mg</td>
<td>321±45***</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>274.8±83.5 g</td>
<td>290.9±71.6</td>
</tr>
<tr>
<td>Total dietary fibers</td>
<td>12.5±4.9 g</td>
<td>13.5±3.0</td>
</tr>
</tbody>
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Data are expressed as means±SD (n=14). ***p<0.001, *p<0.05 compared to Basal by Wilcoxon signed rank test.
Dietary鸡蛋，血清脂质和抗氧化状况

This study was carried out in the autumn of 2013. Due to non-adherence to the protocol, one subject was excluded from the analysis. Fourteen males, most of whom were sedentary workers, with a mean age of 36 y (SD=5.7) and mean body mass index of 22.7 kg/m² (SD=2.8) were enrolled in the analysis. Five subjects had a current smoking habit. Seven subjects had been breakfast skippers before the study. The parameters related to body composition and the values of biochemical tests of renal and liver functions, as well as a hematological test, did not change significantly before and after. Total protein and albumin concentration, however, slightly increased following the study (data not shown).

Dietary analyses

Dairy intake of energy slightly increased during the intervention period, but the difference was not statistically significant (Table 1). The weight of ingested egg during the intervention was almost twice as much as basal (76.5±10.8 g/d and 38.3±20.8 g/d, respectively; p<0.0001). The intake of dietary cholesterol was greater during the intervention compared to the baseline (533±102 mg/d and 379±120 mg/d, respectively; p<0.0001), which was mostly attributable to the additional egg. Although the intake of monounsaturated and polyunsaturated-fatty acids increased, the P/S ratio did not change significantly. Aside from breakfast, there were no appreciable changes in the subjects’ daily nutritional intake (data not shown).

As for alcohol consumption habit, 7, 2, and 5 subjects had drinking habits of 0–1, 2–4, and 5–7 d a week, respectively. One of the subjects continuously took vitamin supplements. During the intervention period, these habits remained unchanged.

Serum lipid profiles

Total cholesterol and LDL-C levels were not altered in the 4-wk period (Table 2). In contrast, HDL-C significantly increased by an average of 6.5% (55.2±11.7 mg/dl to 59.0±15.7 mg/dl, p<0.05), while the L/H cholesterol ratio, a commonly used marker of atherosclerosis risk, decreased from baseline (2.21±0.52 to 2.07±0.70, p<0.05). No significant difference was observed in triglyceride, phospholipids or NEFA. While Apo-A1 level was not increased, the correlation between the change in Apo-A1 and HDL-C was statistically significant (r=0.931, p<0.0001).

Serum antioxidant status

Serum MDA-LDL concentration tended to decrease during the intervention (34.9±4.1 min to 36.7±6.1 min, p=0.19). The serum triglyceride level was significantly prolonged compared to baseline (3.2±0.7 to 4.5±0.87, p=0.05). No significant changes were observed in serum concentrations of lutein + zeaxanthin, vitamin E, or vitamin C (Table 2).

Discussion

In the present study, increased intake of dietary cholesterol from one egg per day for 4 wk did not increase
total plasma or LDL cholesterol in healthy males. A significant increase in HDL-C and a decrease in L/H cholesterol ratio, the major risk marker of CVD, were observed. In addition, a decrease of MDA-LDL and prolonged LDL oxidation lag time indicated that daily moderate egg consumption might reduce LDL oxidizability.

Some studies have shown that dietary cholesterol provided by eggs does not affect cholesterol concentration (10, 11). Others have indicated that egg intake increases both HDL-C and L/C-D and hence, does not affect the L/H cholesterol ratio (12, 13). With a weight loss intervention, consuming 3 eggs per day resulted in no increases in LDL-C while a significant increase in HDL-C was observed in overweight men (14). Bello et al. reported that whole egg consumption improved lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome (15). When lipoprotein particle size and subclasses were analyzed by nuclear magnetic resonance (NMR), significant increases in both LDL and HDL particles as well as an increase in lecithin cholesterol acyltransferase (LCAT) activity were observed by whole egg consumption (16). It has also previously been reported that egg white protein lower the concentration of serum cholesterol by inhibiting cholesterol absorption in animal experiments and clinical trials (17, 18). The lack of the increase of serum cholesterol could be attributed to the relatively low amount of loaded cholesterol and to the alteration of the absorption and reverse transport of cholesterol caused by egg components. The consumption of other food factors which have cholesterol lowering effect, e.g. fiber, soybean, and nuts, stayed unchanged during the intervention period.

Lines of evidence indicate that the principal factor affecting serum cholesterol is not dietary cholesterol but saturated fats (19–21). The Japanese consume diets with low fat (daily energy intake from fat, <30%) and a high ratio of polyunsaturated to saturated fat (P/S), a dietary style recommended for lowering plasma LDL-C levels in western countries. A meta-analysis showed that the response of LDL-C concentrations to changes in dietary cholesterol was weaker in studies with a background diet low in saturated fat (P/S>0.7) than in those with a background diet high in saturated fat (P/S≤0.7) (22). In this study, the dietary P/S ratio in the intervention period (0.72±0.09) was not statistically different from that in the pre-intervention period (0.69±0.20). The lack of the increase of serum cholesterol could be attributed to a dietary style with a high P/S ratio. In fact, Homma et al. reported that the ingestion of high cholesterol diet (+750 mg/d as a dried egg yolk) increased HDL-C, but not TC in 110 Japanese who ingested a low-fat and high P/S ratio diet (23).

In addition to the effects on serum lipids concentrations, we also evaluated the influence of daily egg consumption on serum antioxidant status. We observed a significant decrease in the MDA-LDL/LDL-C ratio, as well as significantly prolonged lag time for LDL oxidation by the egg intervention compared with basal values. Interestingly, plasma TAC value was significantly increased after the study period. As such, there was a negative correlation between the change in MDA-LDL and the changes in TAC (r = −0.623, p < 0.05). On the other hand, serum concentrations of antioxidants such as lutein, zeaxanthin, vitamin E and vitamin C were unchanged. Some intervention trials found the increases in serum lutein and zeaxanthin concentrations after consuming eggs (5, 24). Since we could not find a significant change in the combined amount of serum lutein and zeaxanthin, it would be necessary to measure each concentration in serum and/or in LDL particles. In addition, as eggs contain a high amount of retinol, an antioxidant lipophilic vitamin, subjects’ daily intake of retinol was significantly higher during the intervention period. Although we did not measure serum retinol concentration, it is likely that egg-derived retinol affected serum antioxidant status. These lipophilic antioxidants have been reported to inhibit LDL oxidation in humans (25, 26). Although the direct mechanism is still unclear, the ameliorative effects of egg on serum MDA-LDL level and LDL oxidizability may be attributable to such antioxidants in eggs.

Eggs are among the most nutrient-dense foods regularly consumed in the human diet. In this study, the subjects ate 77 g of egg a day, on average, which accounted for 25% of their daily intake of retinol, and 16%, 20%, 19%, and 60% of those of iron, riboflavin, vitamin D, and cholesterol, respectively. The reliable nutrient intake values from daily dietary records in the present study enabled us to precisely evaluate the nutritional impact of the egg intervention. Adding an egg to the breakfast meal serves to easily improve the nutrient balance, especially in protein, vitamins and minerals.

The open and single-arm design with small sample size of the present study could be limiting factors for the interpretation of results, given that individuals do not uniformly respond to dietary cholesterol. The provided breakfast contained other dietary factors such as vegetables and fruit juice. The effects of smoking and physical activity on serum lipids response and/or oxidative stress markers could not be evaluated. Further studies with a larger number of subjects in a randomised crossover design are needed to assess the effect of egg consumption on cardiovascular health. In this pilot study, additional consumption of one egg per day for 4 wk did not increase either TC or LDL-C in normolipidemic Japanese males. The ameliorative effect of egg consumption on HDL-C and antioxidant status, along with the well-balanced nutritional feature of eggs, might help to reduce atherosclerosis risk.

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