Maintenance of the LDL Cholesterol:HDL Cholesterol Ratio in an Elderly Population Given a Dietary Cholesterol Challenge

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ABSTRACT We previously evaluated the responses to dietary cholesterol in children and young adults. In this study, the effects of dietary cholesterol on plasma lipids and LDL atherogenicity were evaluated in 42 elderly subjects (29 postmenopausal women and 13 men > 60 y old). Our exclusion criteria were diabetes, heart disease, and the use of reductase inhibitors. The study followed a randomized crossover design in which subjects were assigned to consume the equivalent of 3 large eggs (EGG) daily or the same amount of a cholesterol-free, fat-free egg substitute (SUB) for a 1-mo period. After a 3-wk washout period, subjects were assigned to the alternate treatment. The concentration of plasma cholesterol after the EGG period varied among subjects. When all subjects were evaluated, there were significant increases in LDL cholesterol (LDL-C) (P < 0.05) and HDL-C (P < 0.001) for both men and women during the EGG period, resulting in no alterations in the LDL-C:HDL-C or the total cholesterol:HDL-C ratios. In addition, the LDL peak diameter was increased during the EGG period for all subjects. In contrast, the measured parameters of LDL oxidation, conjugated diene formation, and LDL lag time did not differ between the EGG and the SUB periods. We conclude from this study that dietary cholesterol provided by eggs does not increase the risk for heart disease in a healthy elderly population. J. Nutr. 135: 2793–2798, 2005.

KEY WORDS: • dietary cholesterol • LDL-cholesterol • HDL-cholesterol • elderly • LDL size

The elderly represent ~15% of the U.S. population and >83% of the people who die from coronary heart disease are ≥65 y old (1). The physiologic and economical changes, as well as the increased risk of chronic disease, associated with advancing age place the elderly at an interesting crossroad. The nutritional status of many older individuals may lack the balance that is required for optimal health due to risk-reduction interventions that are inappropriate for this population. The basis for the dietary restriction of cholesterol to reduce coronary heart disease (CHD)3 has not been supported by long-term studies in healthy individuals ≥ 65 y old (2). This restriction often fails to provide an adequate level of nutrition and can lead to several nutritional deficiencies in this age group. Adequate nutrition is essential for this population to delay the advent of chronic diseases and to improve the overall quality of life. Eggs can provide older people with a highly nutritious food that is an excellent source of protein, vitamins, and minerals (3); it is also naturally low in SFA as well as high in carotenoids. The American Heart Association’s most recent report recommends that the intake of dietary cholesterol should not exceed 300 mg/d (4). Clearly, the consumption of 1 egg/d would be acceptable if other cholesterol-containing foods were limited. As this segment of the population continues to grow, the burden on the health care industry will become critical. It is important, therefore, to provide the elderly with proper and successful nutritional strategies on health maintenance that can be achieved within their economic and physical capacities.

Epidemiologic studies following subjects for 8 y reported no difference in CHD relative risk between those who consumed <1 egg/wk and those who ate >1 egg/d (5). Data collected from the Lipid Research Clinics Prevalence Follow-Up Study (6), which included men and women (n = 4546), found no association between deaths attributable to CHD and dietary cholesterol consumption. Other studies did not associate elevated total cholesterol (TC) levels, resulting from increased egg consumption, with the incidence of CHD (7–9). It was suggested that the lack of correlation may result from the simultaneous increase in LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) that occurs with egg consumption (10). Because the ratio of LDL-C:HDL-C is a strong predictor of CHD risk, increases in both variables will produce little change in coronary risk.

Another important factor associated with increased CHD is the atherogenicity of the LDL particle. The smaller dense LDL particle, characteristic of pattern B, is considered more atherogenic than the larger buoyant LDL associated with pattern A (11). Smaller LDL particles also have the potential to become more easily oxidized due to their facilitated penetration into the arterial wall, thus increasing the risk for CHD (12).
Meta-analyses have provided data that suggest a modest increase in TC of 0.05–0.06 mmol/L (2–2.2 mg/dL) may be predicted in response to a 100-mg increase in dietary cholesterol (10,11,13). Using this increase as a reference point, individuals whose TC increases > 0.06 mmol/L (2.2 mg/dL) would be classified as hyperresponders to dietary cholesterol. Conversely, those who have no reported change in TC or have increases < 0.05 mmol/L (2 mg/dL) in response to a 100-mg increase in dietary cholesterol, would be classified as hyporesponders.

The existence of the hypo- and hyperresponder classification to dietary cholesterol challenge has been established in various animal models (14,15); we documented that response in children (16), premenopausal women (17), and men aged 20–50 y old (18).

Based on our previous findings (16–18), the main objective of this study was to evaluate the effects of a cholesterol challenge on plasma cholesterol, LDL size, and LDL susceptibility to oxidation in the elderly. Our hypothesis was that the consumption of the equivalent of 3 eggs/d would not increase the risk for CHD in this population.

SUBJECTS AND METHODS

Materials. Liquid whole eggs (EGG) and cholesterol-free, fat-free egg substitute (SUB) were purchased from Vistar. Enzymatic TC and triglyceride (TG) kits were obtained from Roche Diagnostics. Apolipoproteins (apo) B kits, EDTA, aprotinin, copper sulfate, sodium azide, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chemical.

Subjects. Recruitment efforts were conducted through local newspapers and brochure distribution within the surrounding University community. Men were required to be > 60 y old, whereas women were expected to be menopausal for at least 1 y. However, almost 60% of the male responders were taking prescription medication for elevated lipids and were deemed ineligible. This hampered our recruitment of male subjects. Ultimately, 42 healthy volunteers (13 men and 29 women) were recruited with the exclusion criteria for elevated lipids and were deemed ineligible. This hampered almost 60% of the male respondents were taking prescription medication for elevated lipids and were deemed ineligible. This hampered recruitment in children (16), premenopausal women (17), and men aged 20–50 y old (18).

On our previous findings (16–18), the main objective of this study was to evaluate the effects of a cholesterol challenge on plasma cholesterol, LDL size, and LDL susceptibility to oxidation in the elderly. Our hypothesis was that the consumption of the equivalent of 3 eggs/d would not increase the risk for CHD in this population.
TABLE 1
Baseline characteristics of men and women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>197.1 ± 29.3</td>
<td>206.9 ± 47.9</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>114.5 ± 26.8</td>
<td>115.6 ± 46.2</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>51.7 ± 12.0</td>
<td>61.5 ± 14.2</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>91.6 ± 32.0</td>
<td>107.1 ± 57.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 ± 2.9</td>
<td>27.6 ± 5.9</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96.6 ± 9.9</td>
<td>87.3 ± 14.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129.5 ± 9.7</td>
<td>127.8 ± 14.6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84.8 ± 6.6</td>
<td>81.8 ± 7.4</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Use of hormone replacement therapy, n</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. * Different from women, P < 0.05.
2 To convert to mmol/L divide by 38.67.
3 To convert to mmol/L divide by 88.54.

TC, LDL-C, or TG concentrations, blood pressure, or BMI. However, waist circumference was greater in men (P < 0.05) than in women, whereas HDL-C concentrations were greater in women (P < 0.05) than in men. There was 1 woman smoker in the study, and 10 of the 29 postmenopausal women were receiving hormone replacement therapy. The plasma response to dietary cholesterol or dietary intake did not differ between women receiving hormone replacement therapy and those who were not (data not shown).

Consumption of cholesterol, energy from total fat, SFA, and monounsaturated fat (MUFA) was greater during the EGG period than during the SUB period (Table 2). Total energy intake did not differ between the periods, although men had a higher energy intake than women (Table 2). The expected significant increase in dietary cholesterol was also recorded during the EGG period with men having a 635.3 mg/d increase, whereas women reported a 616 mg/d increase (P < 0.05). The percentage of energy obtained from PUFA was not affected. However, the energy derived from carbohydrate and protein was significantly higher during the SUB period (data not shown).

The response of subjects to dietary cholesterol is indicated in Figure 1. According to our previous classification (17,18), we found 27 hypo and 15 hyperresponders to dietary cholesterol. In both men and women, TC (P < 0.05), LDL-C (P < 0.05) and HDL-C (P < 0.001) increased as a result of egg supplementation (Table 3). However, the LDL:HDL ratio and plasma TG did not differ between the EGG and SUB periods. Gender affected the LDL-C:HDL-C ratio (P < 0.05) in that women had a lower average ratio than men in this study, which is associated with the higher concentrations of HDL-C in this group of subjects. A gender effect did not occur with any other variable analyzed.

Despite the increases noted in LDL-C after the dietary cholesterol challenge, apo-B concentration was not affected (Table 4). Concentrations of plasma apo E and apo CIII were also not affected by the dietary treatments (data not shown). The LDL particle diameter did increase during the EGG period compared with the SUB (P < 0.05) (Table 4). However, no changes were observed in the distribution of cholesterol in the different LDL subfractions (Table 4). There were no changes in the parameters of LDL oxidation, conjugated dienes and lag time for men and women during the EGG and SUB periods (data not shown) indicating that the dietary cholesterol challenge did not modify the susceptibility of LDL to oxidation.

Plasma LCAT and CETP activities did not differ between the EGG or SUB periods in either men or women. However, when subjects were categorized as either hyper- or hyporesponders (n = 15) during the EGG and SUB periods.

TABLE 2
Percentage of energy from total fat, SFA, MUFA, and PUFA, and dietary cholesterol of elderly men and women during the EGG or the SUB dietary periods

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total energy</th>
<th>Total fat</th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
<th>Dietary cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kJ/d</td>
<td>% Energy</td>
<td>mg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n = 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGG</td>
<td>8430 ± 2575</td>
<td>37.9 ± 7.0</td>
<td>13.9 ± 3.2</td>
<td>15.2 ± 3.2</td>
<td>7.8 ± 1.9</td>
<td>892.6 ± 135.0</td>
</tr>
<tr>
<td>SUB</td>
<td>9020 ± 2427</td>
<td>34.9 ± 5.9</td>
<td>12.2 ± 2.5</td>
<td>13.5 ± 2.8</td>
<td>6.4 ± 1.8</td>
<td>257.3 ± 127.7</td>
</tr>
<tr>
<td>Women, n = 29</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EGG</td>
<td>8655 ± 4588</td>
<td>43.3 ± 5.5</td>
<td>16.3 ± 2.8</td>
<td>17.4 ± 2.9</td>
<td>8.6 ± 2.5</td>
<td>892.0 ± 190.7</td>
</tr>
<tr>
<td>SUB</td>
<td>8003 ± 4772</td>
<td>38.8 ± 9.3</td>
<td>13.3 ± 3.5</td>
<td>15.7 ± 3.5</td>
<td>8.0 ± 3.5</td>
<td>276.0 ± 302.9</td>
</tr>
<tr>
<td>Repeated-measures ANOVA, P-values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet effect</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Gender effect</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. NS, nonsignificant, P ≥ 0.05.
Repeated-measures ANOVA, \( P \) values

<table>
<thead>
<tr>
<th>Subjects</th>
<th>TC(^2)</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>TG(^3)</th>
<th>LDL-C:HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, ( n = 13 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGG</td>
<td>176.8 ± 30.0</td>
<td>107.4 ± 27.8</td>
<td>51.9 ± 12.9</td>
<td>87.2 ± 34.9</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>SUB</td>
<td>170.8 ± 25.9</td>
<td>105.2 ± 32.4</td>
<td>47.2 ± 12.9</td>
<td>96.5 ± 49.1</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Women, ( n = 29 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGG</td>
<td>195.5 ± 38.8</td>
<td>115.3 ± 40.7</td>
<td>59.2 ± 14.7</td>
<td>104.6 ± 54.7</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>SUB</td>
<td>183.7 ± 34.0</td>
<td>105.2 ± 32.4</td>
<td>57.7 ± 12.0</td>
<td>104.4 ± 59.3</td>
<td>2.0 ± 1.1</td>
</tr>
</tbody>
</table>

Repeated-measures ANOVA, \( P \)-values

- Diet effect: NS, NS, NS, NS, NS
- Gender effect: NS, NS, NS, NS, NS
- Interaction: NS, NS, NS, NS, NS

1 Values are means ± SD. NS, nonsignificant, \( P \geq 0.05 \).
2 To convert to mmol/L divide by 38.67.
3 To convert to mmol/L divide by 88.54.

As expected, the men and women reported a significant increase in the relative amount of total fat, SFA, MUFA, and cholesterol during the egg period, reflecting the difference in nutrient composition between the egg product and the egg substitute. The relative importance of the changes in fat intake vs. dietary cholesterol in determining plasma cholesterol responses cannot be known with certainty, but we believe the latter to be the most important element. The actual increase in plasma cholesterol during the egg period was 0.10 less than the predicted response, assuming a 0.19 mmol/L (7.35 mg/dL) increase for every 1% increase in SFA (9). The lower than expected response in plasma lipids could be due to the hypocholesterolemic effect of dietary MUFA (8), which was higher during the EGG period and could have counterbalanced the increases in SFA observed in the same period. This is why we postulate that the major effects on plasma lipids were due to an excess intake of dietary cholesterol during the EGG period.

**DISCUSSION**

Cholesterol intake often serves as a surrogate marker for 2 other dietary patterns that are associated with increased CHD risk, i.e., high intake of SFA, which results in elevated plasma cholesterol levels, and a dietary pattern that is low in fruits and vegetables, which decreases the intake of B vitamins, antioxidants, and fiber (28). Therefore, analysis of a dietary cholesterol challenge must first consider the possible effects of dietary fats on plasma lipid concentrations.

**Dietary intake analysis.** As expected, the men and women reported a significant increase in the relative amount of total fat, SFA, MUFA, and cholesterol during the egg period, reflecting the difference in nutrient composition between the egg product and the egg substitute. The relative importance of the changes in fat intake vs. dietary cholesterol in determining plasma cholesterol responses cannot be known with certainty, but we believe the latter to be the most important element. The actual increase in plasma cholesterol during the egg period was 0.10 less than the predicted response, assuming a 0.19 mmol/L (7.35 mg/dL) increase for every 1% increase in SFA (9). The lower than expected response in plasma lipids could be due to the hypocholesterolemic effect of dietary MUFA (8), which was higher during the EGG period and could have counterbalanced the increases in SFA observed in the same period. This is why we postulate that the major effects on plasma lipids were due to an excess intake of dietary cholesterol during the EGG period.

**Plasma lipids and dietary cholesterol.** In a study by Schafer et al. (29), increased age was associated with higher LDL-C levels, especially in women. The current study, however, failed to find a gender effect within the LDL-C levels of this elderly population. This is in agreement with studies that document a lack of correlation between gender and plasma lipid response to a dietary cholesterol challenge (30). During the EGG period, both men and women experienced significant increases in LDL-C and HDL-C. The elevated concentrations of both lipoproteins suggest that excess dietary cholesterol is mobilized through the reverse cholesterol transport system in an effort to maintain cholesterol homeostasis. During the EGG period, hyperresponders also experienced a significant increase in LCAT activity, supporting the mobilization of cholesterol...
through the HDL-mediated reverse cholesterol transport pathway.

In the peripheral vascular system, endothelial cells rely on lipoproteins for the transfer of neutral sterols due to an inability to metabolize sterols at this site. Although free cholesterol is transferred to HDL particles through the functioning of a designated HDL receptor (ATP-Binding Cassette A1, ABC-A1), LCAT serves to maintain the concentration gradient toward the HDL core and to preserve the hydrophobic nature of the particle to facilitate transfer (31). The esterification of cholesterol by LCAT produces cholesterol ester (CE), which is concentrated in the HDL core, and may be transferred by CETP in the plasma in exchange for TG. An increase in CETP activity would suggest an enrichment of the apo B lipoproteins in plasma, while simultaneously decreasing HDL-C, and has generally been considered proatherogenic (32). Conversely, if CETP activity is not upregulated, then the decrease in HDL-C does not occur and the particle serves an antiatherogenic function as it returns CE to the liver to be metabolized. This pathway represents a mechanism that may be utilized by individuals in response to a dietary cholesterol challenge to maintain cholesterol homeostasis. Plasma lipid evaluation in other studies suggests that the capacity of HDL to promote cholesterol efflux may be manipulated by diet (33); this offers another explanation for the results of this study.

In this study, both parameters of coronary heart disease risk assessment, LDL-C and HDL-C, increased, yielding no change in the ratio and therefore in risk assessment in the elderly population. There was a significant gender effect for this ratio, however, resulting from the normal elevated HDL levels in women compared with men (34). The increase in LCAT activity in the hyperresponders suggests that, in this population subset, the remodeling of the HDL particle was upregulated to manage the dietary cholesterol challenge. Hyporesponders did not have a significant increase in LCAT, which suggests that an alternate pathway is employed for those whose TC did not increase > 0.06 mmol/L (2.2 mg/dL) for every 100 mg of dietary cholesterol. It is conceivable that hyporesponders may have an upregulation in ABC-A1, resulting in increased cholesterol efflux from the peripheral cells for biliary excretion, and (34,35), decreased synthesis (36) or decreased cholesterol absorption (37) from the intestinal lumen.

Although the LDL:C: HDL-C ratio is an important parameter of risk assessment, so is the atherogeneity of the LDL particle itself. LDL composition varies within the plasma compartment, resulting in a heterogeneous population with respect to size, density, composition, and atherogeneity (38). Those particles that are identified as Pattern B subclass present with a smaller, denser particle and are considered to be more atherogenic than the larger CE-enriched Pattern A subclass (12). The Pattern B subclass has been associated with a 3-fold increase in CHD risk (13,39) and could be related to the ease with which the smaller LDL particle crosses the intima wall of the endothelium and its increased susceptibility to oxidation. Noting a lack of increase in apo B concentration as a result of the dietary cholesterol challenge and the accompanying increase in LDL particle size, we speculate that the number of LDL particles did not increase in this study, but that the plasma response was to create a larger, less atherogenic LDL particle. This speculation is based on the knowledge that each LDL particle contains only one apo B and that the lack of change in apo B concentration during the EGG period indicates that the increase in LDL-C was not due to an increase in the number of circulating particles but to an increase in particle size.

In summary, these results show that postmenopausal women and men > 60 y old with healthy lipoprotein profiles may consume eggs as part of their regular diet. For those subjects who experienced an increase in LDL-C in response to the dietary cholesterol challenge, this was countered by an increase in HDL-C and an increase in the size of the LDL particle (antiatherogenic). In addition, the susceptibility of LDL to oxidation is not enhanced by egg intake.

ACKNOWLEDGMENT

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LITERATURE CITED

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