Maintenance of the LDL Cholesterol:HDL Cholesterol Ratio in an Elderly Population Given a Dietary Cholesterol Challenge[1]

Christine M. Greene, Tosca L. Zern, Richard J. Wood, Sudeep Shrestha, Dimple Aggarwal, Matthew J. Sharman,* Jeff S. Volek,* and Maria Luz Fernandez[2]

*Departments of Nutritional Science and *Kinesiology, University of Connecticut, Storrs, CT 06269*

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).

[1] Supported by the American Egg Board/Egg Nutrition Center.
[2] To whom correspondence should be addressed.
E-mail: maria-luz.fernandez@uconn.edu.
[3] Abbreviations used: ABC-A1, ATP-Binding Cassette A1; apo, apolipoprotein; CE, cholesterol ester; CETP, CE transfer protein; CHD, coronary heart disease; HDL-C, HDL cholesterol; LCAT, lecithin cholesteryl acyl transferase; LDL-C, LDL cholesterol; MUFA, monounsaturated fatty acids; PMSF, phenylmethylsulfonyl fluoride; SUB, substitute; TG, triglycerides.

Manuscript received 27 June 2005. Initial review completed 7 August 2005. Revision accepted 1 September 2005.

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 phenylmethlysulfonyl 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 respondents 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 consisting of *1*) allergy to eggs; *2*) lipid-lowering medication; *3*) history of heart disease, diabetes, or kidney problems; *4*) total cholesterol concentrations > 6.2 mmol/L (240 mg/dL) or total TG levels > 3.4 mmol/L (300 mg/dL).

Experimental protocol. Subjects gave their informed consent to participate in the intervention and all protocols were approved by the University of Connecticut Institutional Review Board. This study utilized a randomized crossover design in which subjects were initially assigned to an EGG or SUB group for 30 d, followed by a 3-wk washout period. The subjects were then crossed over to the alternate dietary intervention and continued for 30 d. Those assigned to the EGG group were expected to consume the egg product provided, which was the equivalent of 3 eggs/d (~640 mg dietary cholesterol). Conversely, the SUB group was given an equal volume of a cholesterol-free, fat-free product almost identical in color and consistency to the egg product (0 mg dietary cholesterol). Daily amounts were provided in individual containers and subjects were instructed to return any uneaten portion at the beginning of the following week.

Subjects were advised to avoid additional egg consumption outside of that provided by the study and to maintain their regular diets. To ensure that diets during the intervention periods were equivalent, two 7-d dietary records were collected. Records included 2 weekend days and 5 nonconsecutive weekdays. Nutrient intake was evaluated using Nutritional Data Systems software (NDS-R) Version 5.0, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 29.

Two blood draws from subjects who had fasted for 12 h were scheduled at the end of each intervention period, on nonconsecutive days, 2 d apart, for each subject. Whole blood was collected into tubes containing 0.10 g/100 g EDTA to determine plasma lipids. Plasma was separated by centrifugation at 1500 × *g* for 20 min at 4°C, and placed into vials containing PMSF (0.05 g/100 g), sodium azide (0.01 g/100 g), and aprotinin (0.01 g/100 g). The variables of waist and hip circumference and weight were also measured at baseline and at the end of each dietary period to account for the potential influence of these factors on plasma lipid levels.

Plasma lipids and apolipoproteins. Our laboratory has participated in the Center for Disease Control/National Heart, Lung, and Blood Institute (CDC-NHLBI) Lipid Standardization Program since 1989 for quality control and standardization for plasma TC, HDL-C, and TG assays. The CVs assessed by the Standardization program during the last human study were 0.76–1.42% for TC, 1.71–2.72% for HDL-C, and 1.64–2.47% for TG. The effects of a dietary cholesterol challenge on the TC, LDL-C, HDL-C, and TG concentrations and the LDL-C:HDL-C ratio were examined. TC was determined by enzymatic methods (19), whereas HDL-C was measured in the supernatant after precipitation of apo-B–containing lipoproteins (20). LDL-C was determined using the Friedewald equation (21), and TG concentrations were determined using Roche-Diagnostics kits, which adjust for free glycerol (22). The means of the 2 blood draws were used to analyze the variances between treatment periods. Apo B analysis was performed using Sigma Chemical kits, which utilize immunoturbidity to ascertain concentration. Turbidity was measured in a microplate spectrophotometer at 340 nm (23). Apo C-III and apo E were measured on a Hitachi Autoanalyzer 740 with Wako kits used according to the manufacturer's instructions.

LDL size. The Lipoprint LDL system (Quantimetrix), which is a nongradient high-resolution PAGE method, was used to determine LDL particle size as previously reported (24). To analyze the sample, 25 μL of plasma was added to a prefilled polyacrylamide gel tube and overlaid with 200 μL of loading gel. Tubes were then photopolymerized for 30 min and placed into the electrophoresis chamber. Electrophoresis buffer (Tris-hydroxymethyl aminomethane 66.1 g/100 g, boric acid 33.9 g/100 g, pH solution 8.2–8.6) was added to the top and bottom portion of the chamber. Bands for LDL size were quantified with a densitometer.

LDL oxidation. LDL lag-time and diene production were determined according to Abbey et al. (25). Briefly, LDL was isolated by ultracentrifugation in a L8-mol/L ultracentrifuge at a density of 1.09 kg/L at 65,000 × *g* for 45 min. Samples were dialyzed overnight and LDL protein was determined by the Lowry method (26). Kinetics of the samples were followed at 37°C, for 180 min, and absorbance was plotted every 120 s. The rate of diene production was determined from the slope of the propagation phase. Lag time was determined from the intercept of the lag and propagation phases.

Plasma lecithin cholesteryl acyl transferase (LCAT) and cholesteryl ester transfer protein (CETP). Physiologic CETP activity was determined without inhibition of LCAT action according to the calculations described by Ogawa and Fielding (27). LCAT activity was determined by the mass analysis of the decrease in plasma free cholesterol between 0 and 6 h at 37°C.

Classification of hyper- and hyporesponders. As previously described, a modest increase in TC of 0.05–0.06 mmol/L (2–2.0 mg/dL) may be considered a normal response to a 100-mg dietary cholesterol challenge. Therefore, in this study, participants who experienced an increase in TC ≥ 0.06 mmol/L (2.2 mg/dL) for each additional 100 mg cholesterol ingested were considered hyperresponders. The subjects consumed an additional 640 mg/d of dietary cholesterol and those that had an increase in TC of ≥0.41 mmol/L (16 mg/dL) were labeled hyperresponders in this study. Those subjects whose fluctuations in TC were ≤0.36 mmol/L (14 mg/dL), were identified as hyporesponders (16–18).

Statistical analysis. Repeated-measures ANOVA was used to analyze the effects of dietary cholesterol on plasma lipids, lipoprotein metabolism, and LDL particle characteristics. Each individual's response to diet (EGG or SUB) was considered to be the repeated measure; gender was the between-subject factor. Differences with $P < 0.05$ were considered significant. Data are presented as means ± SD for the number of subjects in each group. Statistical analysis was conducted using SPSS v. 12 for Windows.

RESULTS

Baseline characteristics of the participants are shown in **Table 1**. Men and women did not differ at baseline in plasma

TABLE 1

Baseline characteristics of men and women[1]

Variable	Men	Women
n	13	29
TC,[2] *mg/dL*	197.1 ± 29.3	206.9 ± 47.9
LDL-C, *mg/dL*	114.5 ± 26.8	115.6 ± 46.2
HDL-C, *mg/dL*	51.7 ± 12.0*	61.5 ± 14.2
TG,[3] *mg/dL*	91.6 ± 32.0	107.1 ± 57.3
BMI, *kg/m²*	26.1 ± 2.9	27.6 ± 5.9
Waist circumference, *cm*	96.6 ± 9.9*	87.3 ± 14.6
Systolic blood pressure, *mm Hg*	129.5 ± 9.7	127.6 ± 14.6
Diastolic blood pressure, *mm Hg*	84.8 ± 6.6	81.8 ± 7.4
Smokers, *n*	0	1
Use of hormone replacement therapy, *n*	—	10

[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.

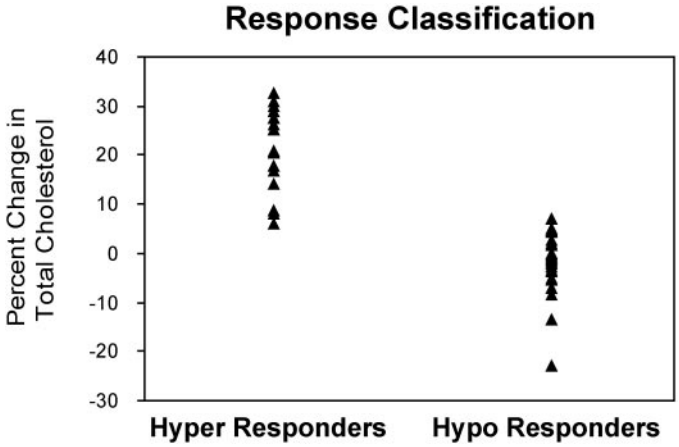

FIGURE 1 Percentage change in plasma TC in hypo- ($n = 27$) and hyper-responders ($n = 15$) during the EGG and SUB periods.

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 hypore-

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[1]

Subjects	Total energy	Total fat	SFA	MUFA	PUFA	Dietary cholesterol
	kJ/d	*% Energy*				*mg/d*
Men, $n = 13$						
EGG	8430 ± 2575	37.9 ± 7.0	13.9 ± 3.2	15.2 ± 3.2	7.8 ± 1.9	892.6 ± 135.0
SUB	9020 ± 2427	34.9 ± 5.9	12.2 ± 2.5	13.5 ± 2.8	6.4 ± 1.8	257.3 ± 127.7
Women, $n = 29$						
EGG	8655 ± 4588	43.3 ± 5.5	16.3 ± 2.8	17.4 ± 2.9	8.6 ± 2.5	892.0 ± 190.7
SUB	8003 ± 4772	38.8 ± 9.3	13.3 ± 3.5	15.7 ± 3.5	8.0 ± 3.5	276.0 ± 302.9
Repeated-measures ANOVA, *P*-values						
Diet effect	NS	<0.05	<0.001	<0.005	NS	<0.0001
Gender effect	<0.05	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS

[1] Values are means ± SD. NS, nonsignificant, $P \geq 0.05$.

TABLE 3

Plasma TC, LDL-C, HDL-C, and TG of men and women after the EGG or SUB periods[1]

Subjects	TC[2]	LDL-C	HDL-C	TG[3]	LDL-C:HDL-C
			mg/dL		
Men, $n = 13$					
EGG	176.8 ± 30.0	107.4 ± 27.8	51.9 ± 12.9	87.2 ± 34.9	2.2 ± 1.1
SUB	170.8 ± 25.9	105.2 ± 32.4	47.2 ± 12.9	96.5 ± 49.1	2.4 ± 1.1
Women, $n = 29$					
EGG	195.5 ± 38.8	115.3 ± 40.7	59.2 ± 14.7	104.6 ± 54.7	2.2 ± 1.1
SUB	183.7 ± 34.0	105.2 ± 32.4	57.7 ± 12.0	104.4 ± 59.3	2.0 ± 1.1
Repeated-measures ANOVA, *P*-values					
Diet effect	<0.05	<0.05	<0.001	NS	NS
Gender effect	NS	NS	NS	NS	<0.05
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.

sponders to dietary cholesterol, LCAT activity was greater in the hyperresponders as a result of the dietary cholesterol challenge [42.0 ± 29.5 vs. 31.1 ± 20.0 nmol/(h · L plasma)] (**Fig 2**).

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

TABLE 4

Plasma cholesterol in the LDL subfractions, LDL diameter, and apo B Concentrations[1]

Subjects	Plasma apo B	LDL-1[2]	LDL-2	LDL-3	LDL Diameter
	mg/L		*mg/dL*		*nm*
Men, $n = 13$					
EGG	796 ± 147	60.2 ± 20.7	18.9 ± 8.0	13.0 ± 8.7	26.2 ± 1.2
SUB	847 ± 183	61.5 ± 18.5	15.6 ± 8.2	12.9 ± 9.9	25.7 ± 1.2
Women, $n = 29$					
EGG	879 ± 208	60.2 ± 20.8	18.3 ± 10.2	12.9 ± 13.1	26.1 ± 1.4
SUB	799 ± 177	60.9 ± 26.5	17.8 ± 7.8	15.0 ± 18.0	25.8 ± 1.8
Repeated-measures ANOVA, *P*-values					
Diet effect	NS	NS	NS	NS	<0.05
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.

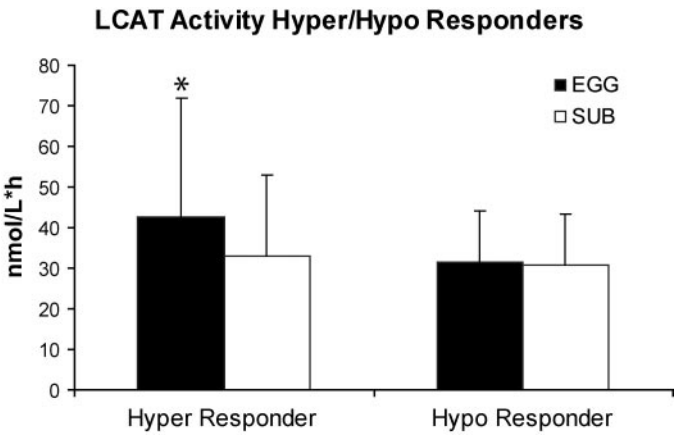

FIGURE 2 Plasma LCAT activity [nmol/(L · h)] in hyper- and hyporesponders during the EGG and SUB periods. *Different from SUB, $P < 0.05$.

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 compartment to apo B–containing lipoproteins 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 elder 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 atherogenicity of the LDL particle itself. LDL composition varies within the plasma compartment, resulting in a heterogeneous population with respect to size, density, composition, and atherogenicity (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

We acknowledge the time and effort of our phlebotomist, Brian O'Neil.

LITERATURE CITED

1. Bureau USC. United States Census 2000. 2000:www.census.gov.
2. Krumholz HM, Seeman TE, Merrill SS, Mendes de Leon CF, Vaccarino V, Silverman DI, Tsukahara R, Ostfeld AM, Berkman LF. Lack of association between cholesterol and coronary heart disease mortality and morbidity and all-cause mortality in persons older than 70 years. J Am Med Assoc. 1994;272:1335–40.
3. NewZealand Egg Board. Egg Nutrition. The Incredible Edible Egg. The Amino Acid Content of Foods and Biological Data on Proteins. Nutritional Study #24. Rome: Food and Agriculture Organization of the United Nations, 1998: www.eggs.org.nz/nutrition.htm.
4. US Dept of Health and Human Services H. Dietary Guidelines 2005. My Pyramid 2005. Washington, DC: USDA, 2005.
5. Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascherio A, Colditz GA, Rosner BA, Spiegelman D, Speizer FE, Sacks FM, Hennekens CH, Willett WC. A prospective study of egg consumption and risk of cardiovascular disease in men and women. J Am Med Assoc. 1999;281:1387–94.
6. Esrey KL, Joseph L, Grover SA. Relationship between dietary intake and coronary heart disease mortality: lipid research clinics prevalence follow-up study. J Clin Epidemiol. 1996;49:211–6.
7. Dawber TR, Nickerson RJ, Brand FN, Pool J. Eggs, serum cholesterol, and coronary heart disease. Am J Clin Nutr. 1982;36:617–25.
8. Fraser GE. Diet and coronary heart disease: beyond dietary fats and low-density-lipoprotein cholesterol. Am J Clin Nutr. 1994;59:1117S–23.
9. Gramenzi A, Gentile A, Fasoli M, Negri E, Parazzini F, La Vecchia C. Association between certain foods and risk of acute myocardial infarction in women. Br Med J. 1990;300:771–3.
10. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. Br Med J. 1997;314:112–7.
11. 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.
12. Krauss RM. Is the size of low-density lipoprotein particles related to the risk of coronary heart disease? J Am Med Assoc. 2002;287:712–3.
13. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. J Am Med Assoc. 1996;276:875–81.
14. Eggen DA. Cholesterol metabolism in groups of rhesus monkeys with

high or low response of serum cholesterol to an atherogenic diet. J Lipid Res. 1976;17:663–73.

15. Roberts DC, West CE, Redgrave TG, Smith JB. Plasma cholesterol concentration in normal and cholesterol-fed rabbits. Its variation and heritability. Atherosclerosis. 1974;19:369–80.

16. Ballesteros MN, Cabrera RM, Saucedo Mdel S, Fernandez ML. Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population from northern Mexico. Am J Clin Nutr. 2004;80:855–61.

17. Herron KL, Vega-Lopez S, Conde K, Ramjiganesh T, Roy S, Shachter NS, Fernandez ML. Pre-menopausal women, classified as hypo- or hyperresponders, do not alter their LDL/HDL ratio following a high dietary cholesterol challenge. J Am Coll Nutr. 2002;21:250–8.

18. Herron KL, Vega-Lopez S, Conde K, Ramjiganesh T, Shachter NS, Fernandez ML. Men classified as hypo- or hyperresponders to dietary cholesterol feeding exhibit differences in lipoprotein metabolism. J Nutr. 2003;133:1036–42.

19. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20:470–5.

20. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem. 1982;28:1379–88.

21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.

22. Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem. 1993;26:39–42.

23. Rifai N, Silverman LM. Immunoturbidimetric techniques for quantifying apolipoproteins CII and CIII. Clin Chem. 1986;32:1969–72.

24. Herron KL, Lofgren IE, Sharman M, Volek JS, Fernandez ML. High intake of cholesterol results in less atherogenic low-density lipoprotein particles in men and women independent of response classification. Metabolism. 2004;53:823–30.

25. Abbey M, Belling GB, Noakes M, Hirata F, Nestel PJ. Oxidation of low-density lipoproteins: intraindividual variability and the effect of dietary linoleate supplementation. Am J Clin Nutr. 1993;57:391–8.

26. Markwell MA, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem. 1978;87:206–10.

27. Ogawa Y, Fielding CJ. Assay of cholesteryl ester transfer activity and purification of a cholesteryl ester transfer protein. Methods Enzymol. 1985;111:274–85.

28. McNamara DJ. Dietary cholesterol and atherosclerosis. Biochim Biophys Acta. 2000;1529:310–20.

29. Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP, Wilson PW. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res. 1994;35:779–92.

30. Katan MB, Beynen AC, de Vries JH, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol. 1986;123:221–34.

31. Jonas A. Lecithin cholesterol acyltransferase. Biochim Biophys Acta. 2000;1529:245–56.

32. Tall AR. Plasma lipid transfer proteins. J Lipid Res. 1986;27:361–7.

33. Blanco-Molina A, Castro G, Martin-Escalante D, Bravo D, Lopez-Miranda J, Castro P, Lopez-Segura F, Fruchart JC, Ordovas JM, Perez-Jimenez F. Effects of different dietary cholesterol concentrations on lipoprotein plasma concentrations and on cholesterol efflux from Fu5AH cells. Am J Clin Nutr. 1998;68:1028–33.

34. Nestel PJ, Poyser A. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism. 1976;25:1591–9.

35. Quintao EC, Sperotto G. The role of dietary cholesterol in the regulation of human body cholesterol metabolism. Adv Lipid Res. 1987;22:173–88.

36. Mistry P, Miller NE, Laker M, Hazzard WR, Lewis B. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. Studies of low density lipoprotein receptor activity and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in blood mononuclear cells. J Clin Invest. 1981;67:493–502.

37. Sehayek E, Ono JG, Shefer S, Nguyen LB, Wang N, Batta AK, Salen G, Smith JD, Tall AR, Breslow JL. Biliary cholesterol excretion: a novel mechanism that regulates dietary cholesterol absorption. Proc Natl Acad Sci U S A. 1998;95:10194–9.

38. Sevanian A, Asatryan L, Ziouzenkova O. Low density lipoprotein (LDL) modification: basic concepts and relationship to atherosclerosis. Blood Purif. 1999;17:66–78.

39. Austin MA. Genetic and environmental influences on LDL subclass phenotypes. Clin Genet. 1994;46:64–70.