# High Intake of Cholesterol Results in Less Atherogenic Low-Density Lipoprotein Particles in Men and Women Independent of Response Classification

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**The influence of a high-cholesterol diet on the atherogenicity of the low-density lipoprotein (LDL) particle was examined by measuring LDL peak diameter and composition, LDL susceptibility to oxidation, and the distribution of cholesterol between LDL subclasses. The crossover intervention randomly assigned 27 premenopausal women and 25 men (18 to 50 years) to an egg (640 mg/d additional dietary cholesterol) or placebo (0 mg/d additional dietary cholesterol) diet for 30 days, followed by a 3-week washout period. Subjects were classified as either hyperresponders (>2.5 mg/dL increase in plasma cholesterol for each 100 mg additional dietary cholesterol consumed) or hyporesponders to dietary cholesterol. Sex was found to have a significant effect on 3 of the parameters examined. LDL peak diameter was significantly larger ($P < .005$) in females (26.78 ± 0.59 nm, n = 27) as compared with males (26.52 ± 0.49 nm, n = 25), regardless of response to dietary cholesterol. The LDL particles of the male participants also had a higher number of triglyceride (TG) and cholesteryl ester (CE) molecules ($P < .01$); however, cholesterol ester transfer protein (CETP) activity was higher in females ($P < .05$). Response classification also revealed significant differences in the determination of LDL subclasses. Independent of sex, the LDL-1 particle ($P < .05$), which is considered to be less atherogenic, was predominant in hyperresponders and this finding was associated with increased cholesterol intake (interactive effect, $P < .001$). In addition, CETP and lecithin: cholesterol acyltransferase (LCAT) activities were higher in hyperresponders during the egg period (interactive effect, $P < .05$). Sex, response to cholesterol intake, and diet were not found to affect the susceptibility of LDL to oxidation ($P > 0.5$). Because LDL peak diameter was not decreased and the larger LDL-1 subclass was greater in hyperresponders following egg intake, these data indicate that the consumption of a high-cholesterol diet does not negatively influence the atherogenicity of the LDL particle.**
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CURRENT DIETARY recommendations state that egg intake should be limited and that dietary cholesterol consumption above 300 mg/d has a significant negative influence on plasma total cholesterol (TC) concentrations. These recommendations are based on the assumptions that all persons experience plasma fluctuations following intake of dietary cholesterol and that TC elevations translate directly to increased risk for the development of atherosclerosis and coronary heart disease (CHD). Because CHD is the leading cause of death in the United States,[1] it is important to examine variations in the individual response to dietary cholesterol and to determine whether egg intake influences risk factors, such as the atherogenicity of the low-density lipoprotein (LDL) particle, which have been identified as important predictors of disease.

It is clear that the composition of the diet influences lipoprotein concentration, composition, and metabolism,[2,3] which may affect the development of atherosclerosis and CHD. The association between elevated LDL-cholesterol (LDL-C) and an increased risk for CHD has been well documented.[4] However, LDL particles are heterogeneous with regard to size, density, composition, charge, and atherogenicity.[5] Based on their size, these particles have been identified as LDL-1 through LDL-7. In this classification, larger numbers indicate a decrease in peak particle diameter. A predominance of small, dense LDL particles (ie, LDL-3+), which are considered to be more atherogenic than the larger more buoyant cholesteryl ester (CE) enriched fraction (Pattern A subclass),[6] would be representative of the Pattern B subclass. This LDL subclass has been shown to be associated with a 3-fold increase in CHD risk,[7,8] which may be due to the easy entry of the smaller particles into the arterial wall,[9] their enhanced binding to the proteoglycans,[10] and their increased susceptibility to oxidation.[6,8] This enhanced susceptability may be due to lowered tocopherol content[11] or increased polyunsaturated fat concentration[12] in the more dense LDL subclass. Furthermore, oxidized LDL possesses an increased atherogenicity due to its unregulated uptake by macrophages and its role in foam cell production. In addition, the smaller lipoprotein particles have been shown to have decreased affinity for the LDL receptor,[13,14] which would result in increased plasma half-life that may enhance LDL anchorage to the arterial wall.[15]

If egg intake does indeed have negative health implications, as current recommendations suggest, consumption would be expected to result in the development of a more atherogenic LDL particle. Therefore, the main objective of this study was to determine LDL particle size and composition, susceptibility of the particle to oxidation, and the distribution of cholesterol across LDL subclasses in men and women classified as hyper- and hyporesponders to a diet high in cholesterol.

## MATERIALS AND METHODS

### *Materials*

Liquid pasteurized whole eggs and cholesterol-free/fat-free eggs (placebo) were purchased from Better Brands (Windsor, CT). Enzymatic cholesterol, and triglyceride (TG) kits were obtained from

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Roche-Diagnostics (Indianapolis, IN). EDTA, phospholipids, and free cholesterol kits were obtained from Wako Pure Chemical (Osaka, Japan); aprotinin, sodium azide, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chemical (St Louis, MO). Malonaldehyde bis (diethyl acetal) was obtained from Aldrich (Arlington Heights, IL). Human insulin specific radioimmunoassay (RIA) kit was from Linco Research (St Charles, MO).

### *Subjects*

A total of 40 men and 51 premenopausal women participated in the dietary intervention.[16,17] Of this population, plasma samples from a subset of men (n = 25) and premenopausal women (n = 27) were used for the analysis reported in this study. Subjects were recruited from the University community and were between the ages of 20 and 50 years. The exclusion criteria for this study included the presence of hypercholesterolemia (cholesterol >240 mg/dL), hypertriglyceridemia (TGs > 300 mg/dL), hypertension, and diabetes. Furthermore, those receiving lipid-lowering drugs were also excluded.

### *Experimental Design*

The experimental protocol was approved by the University of Connecticut's Institutional Review Board, and written informed consent was obtained from each subject. The study utilized a randomized crossover design, with subjects initially assigned to an egg or placebo group for 30 days, followed by a 3-week washout period, after which the second dietary period began. Subjects assigned to the egg group were expected to consume the liquid equivalent of 3 whole eggs per day (adding approximately 640 mg/d cholesterol to the diet). In contrast, those assigned to the placebo consumed an identical weight of cholesterol-free and fat-free egg substitute (0 mg/d dietary cholesterol). Both products were identical in terms of color and consistency and differed only in the fat and cholesterol content. Daily portions were provided in individual containers, and subjects were asked to return any uneaten portion at the end of the week.

Subjects were expected to adhere to the National Cholesterol Education Program (NCEP) step I diet for the duration of the study, and detailed dietary instructions were provided. The NCEP step I diet recommends that no more than 30% of total energy come from fat, with saturated fat providing only 10% of the total. In addition, subjects were instructed to consume no more than 300 mg/d of dietary cholesterol in their self-selected diet. To ensure compliance with the dietary guidelines, subjects completed seven 24-hour dietary records during each treatment period, which included 2 weekend days. Nutrient intake was determined using the Nutrition Data System for Research (NDS-R) software version 4.0, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN.

Two fasting (12 hour) blood samples were initially collected, on different days within the same week, into tubes containing 0.15 g/100 g EDTA to determine baseline plasma lipids. Plasma was separated by centrifugation at 1,500 × *g* for 20 minutes at 4°C and placed into vials containing PMSF (0.05 g/100g), sodium azide (0.01 g/100 g) and aprotinin (0.01 g/100 g). Two additional blood samples were collected and processed in the same manner at the end of each diet treatment and washout period. The variables of weight, blood pressure, level of activity, smoking, and alcohol intake were also measured at baseline and after each dietary period to account for the possible influence of these factors on plasma lipid levels and lipoprotein metabolism.

### *Plasma Lipids*

TC was determined by enzymatic methods using Roche-Diagnostics standards and kits.[18] High-density lipoprotein-cholesterol (HDL-C) was measured in the supernatant after precipitation of apolipoprotein (apo) B-containing lipoproteins[19] and LDL-C was determined using the Friedewald equation.[20] TG were determined using Roche-Diagnostics kits, which adjust for free glycerol. Our laboratory has been participating in the Centers 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. Coefficients of variance assessed by the Standardization program during the study period were 0.76 to 1.42 for TC, 1.71 to 2.72 for HDL-C, and 1.64 to 2.47 for TG.

### *Classification of Hyper- and Hyporesponders*

As previously mentioned, a modest increase in TC of 2.2 to 2.5 mg/dL may be considered normal in response to a 100-mg increase in dietary cholesterol. For the purpose of this study, subjects who experienced an increase in TC ≥ 2.5 mg/dL for each additional 100 mg of dietary cholesterol consumed were considered hyperresponders.[16,17] Because the subjects were fed an additional 640 mg/d of dietary cholesterol (approximately 213 mg/large egg) during the egg period, those who experienced an increase in TC of ≥16 mg/dL were considered hyperresponders. The remaining subjects who experienced fluctuations of <14 mg/dL (an increase in TC of 2.2 mg/dL for every 100 mg of additional dietary cholesterol consumed) or had no change in TC were identified as hyporesponders. The reproducibility of individual differences in response has been previously documented in several controlled and field trials.[21]

### *Plasma Cholesterol Ester Transfer Protein and Lecithin Cholesterol Acyltransferase Activities*

Plasma cholesterol ester transfer protein (CETP) activity was determined in plasma according to the method described by Ogawa and Fielding.[22] This method measures the mass transfer of CE between HDL and apo B containing-lipoproteins. Thus, physiologic CETP activity was assessed through an analysis of the decrease in HDL CE mass between 0 and 6 hours, without lecithin cholesterol acyltransferase (LCAT) inhibition. Samples were incubated at 37°C for 6 hours in a shaking water bath. Following this period, total, HDL, and free plasma cholesterol were measured, and previously described calculations were performed.[23] LCAT activity was determined by an endogenous self-substrate method, which involves mass analysis of the decrease in plasma free cholesterol between 0 and 6 hours at 37°C. Assays were performed concurrently with measurements of CETP. Both of these methods have been standardized in our laboratory.

### *Plasma Insulin*

Insulin was measured in plasma using a RIA kit that utilizes the double-antibody/PEG technique.[24] Briefly, 100 $\mu$L plasma was incubated with $^{125}$I-labeled human insulin and guinea pig antihuman insulin antiserum. After an overnight incubation, a precipitating reagent containing goat antiguinea pig immunoglobulin G (IgG) was added and samples were mixed and incubated for 20 minutes. Samples were then centrifuged at 2,500 × *g* for 20 minutes, after which the liquid was decanted, and tubes containing the resulting pellet were each counted for 1 minute using a Cobra II-Auto Gamma Counting System (Packard Instruments, Meriden, CT).

### *LDL Isolation and Characterization*

LDL was isolated by sequential ultracentrifugation in an LE-80K ultracentrifuge (Beckman Instruments, Palo Alto, CA) for 45 minutes at 200,000 × *g* and 15°C, using a Ti-65 vertical rotor, as previously described.[25] The isolated lipoprotein samples were then dialyzed overnight (0.01% $Na_2EDTA$, 0.9% NaCl pH 7.2 to 7.4) at 4°C.

LDL composition was calculated after the concentrations of the main components, free cholesterol (FC),[26] CE,[27] TG,[28] phospholipids (PL), and protein, had been determined. TC, FC, TG, and PL concentrations

were measured using enzymatic methods. Esterified cholesterol was calculated by subtracting FC from TC. Protein concentration was measured by a modified Lowry procedure.[29] The number of component molecules of LDL was calculated assuming 1 apo B molecule (molecular weight 550 kd) per particle. The molecular weights of TG, FC, CE, and PL used were 885.4, 386.6, 664, and 734, respectively.[30]

### *LDL Size Determination*

The Lipoprint LDL system (Quantimetrix Redondo Beach, CA), which utilizes nongradient high-resolution polyacrylamide gel electrophoresis, was used to determine LDL peak particle diameter and subclass distribution. Briefly, 25 $\mu$L plasma was added to precast polyacrylamide gel tubes and overlayed with 200 $\mu$L loading buffer. Tubes were then photopolymerized for approximately 30 minutes and then placed into the electrophoresis chamber. Electrophoresis buffer (Tris-hydroxymethyl aminomethane 66.1 g/100 g, boric acid 33.9 g/100 g, pH 8.2 to 8.6) was added to the top and bottom portion of the chamber. The gel was run for approximately 60 minutes at 36 mV or until the HDL fraction was approximately 1 cm from the end of the tube. Gels were allowed to sit for 30 minutes and then scanned with a densitometer. The Lipoprint system quantifies 6 different LDL subclasses based on size. The majority of subjects did not have LDL-4, -5, and -6 in an amount that could be detected; therefore, only 3 fractions are reported here. However, for those subjects who carried a detectable amount of cholesterol in the smaller LDL fractions, these concentrations were added into LDL-3 fraction.[31]

### *LDL Susceptibility to Oxidation*

The apo B-containing lipoprotein fraction, consisting of very–low-density lipoprotein (VLDL,) intermediate-density lipoprotein (IDL), and LDL, was isolated from plasma by ultracentrifugation in an LE-80K ultracentrifuge (Beckman Instruments, Palo Alto, CA) for 45 minutes at 200,000 × $g$ and 15°C, using a Ti-65 vertical rotor.[25] Separation was based on $d$ = 1.063 mg/mL. Samples were dialyzed overnight in an EDTA-free phosphate buffered saline (PBS; 10 nmol/L $NaH_2PO_4$, 0.15 mol/L NaCl, pH 7.4), at 4°C. In vitro LDL susceptibility to oxidation was determined by the measurement of the formation of thiobarbituric acid reactive substances (TBARS), after copper-mediated oxidation of the apo B-containing lipoprotein fraction, as previously reported.[23] The lipid peroxide content was expressed as malondialdehyde equivalents.

### *Data Analysis*

A 3-way repeated measures analysis of variance (ANOVA) was used to analyze diet, sex, and response effects on the characteristics of the LDL particle with each subject during the egg or palcebo period as the repeated measure. Significant interactions were detected by LSD protected test, and $P < .05$ was considered significant.

## RESULTS

Of the 91 subjects who completed the study, 28 (14 women and 14 men) hyper- and 26 (13 women and 13 men) hyporesponders to dietary cholesterol were selected for the LDL particle analysis. No significant differences existed within this subset between dietary periods; however, men did have significantly ($P < .001$) higher body mass index (BMI) values (25.5 ± 3.2 kg/$m^2$, n = 25) than women (22.6 ± 3.3 kg/$m^2$, n = 27). As previously reported,[16,17] an analysis of the 7-day dietary records revealed that all subjects complied with the requirements of the NCEP step I diet. A significantly greater average intake of 764.3 ± 67.1 mg/d of dietary cholesterol ($P < .0001$) was reported during the egg as compared with the average 166.9 ± 118.3 mg/d consumed during the placebo period. In addition, the average contribution of energy derived from total (31.4% ± 5.5%) and saturated fat (10.9% ± 2.4%) during the egg period was significantly higher ($P < .01$) than total (26.6% ± 7.1%) and saturated fat (9.4% ± 2.6%) during the placebo period.

Plasma insulin was measured in these subjects to determine whether dietary treatment would affect this hormone and influence the characteristics of the LDL during the egg or placebo periods. There were no significant differences in insulin due to diet or individual response (data not shown). However, plasma insulin levels were higher ($P < .05$) in men (13.0 ± 5.7 $\mu$U/L, n = 25) when compared with women (9.0 ± 7.3 $\mu$U/L, n = 27). There was also a significant correlation between BMI and plasma insulin for all subjects ($r$ = .601, $P < .0001$).

### *The Effect of Diet, Sex, and Response on LDL Phenotype, Subclass Distribution, and Peak Diameter*

Of the men and women studied, 29 were classified as having the pattern B phenotype during the placebo period, while 25 were pattern A. During the egg period, all participants were equally distributed (27 in each group) between the 2 phenotypes. This indicates a nonsignificant shift of 5 participants from pattern B to A and 4 participants from pattern A to B following egg consumption (data not shown). The larger LDL subclass was determined to be more prominent in women when compared with men (Table 1). Furthermore, women had higher concentrations of LDL-1 regardless of response classification. However, independent of sex, hyperresponders were found to have significantly ($P < .05$) larger concentrations of the LDL-1 subclass than their hypo-responsive counterparts. (Table 1). Furthermore, the analysis of LDL subclass indicated that a significant ($P < .001$) interactive effect existed between diet and response, which indicated that hyperresponders had the greatest level of LDL-1 following egg consumption. In contrast to LDL-1, the distribution of cholesterol in LDL-2 was not independently influenced by sex, dietary treatment, or response to dietary cholesterol (Table 1). However, an interaction between sex, diet, and response ($P < .01$) was found with regard to LDL-2, which showed that this particle was elevated in female hyperresponders following egg consumption and following intake of the placebo for male hyperresponders. The distribution of cholesterol in the smallest LDL subfraction reported was influenced by sex, with the male population having a greater concentration of LDL-3 when compared with women (Table 1). Furthermore, LDL-3 was also highest in men during the egg as compared with the placebo period. The LDL peak particle diameter was significantly ($P < .01$) larger in women than men (Table 1); however, it was affected by an interaction with response. Women classified as hyperresponders had larger peak LDL diameter when compared with hyporesponders. In contrast, male hyporesponders had larger LDL peak diameter than hyperresponders (Table 1).

### *The Effect of Diet, Sex, and Response on LDL Oxidation and the Activities of LCAT and CETP*

Based on response classification, LDL-C concentrations were significantly higher in hyperresponders following egg

**Table 1. Distribution of Cholesterol in LDL Subclasses and LDL Peak Diameter of Hypo- and Hyper-responders During the EGG or SUB Periods**

| | LDL-1 (mg/dL) | LDL-2 (mg/dL) | LDL-3 (mg/dL) | LDL Peak Diameter (nm) |
|---|---|---|---|---|
| Women | | | | |
| Hyper-responders (n = 14) | | | | |
| EGG | 31.7 ± 12.2[a] | 22.1 ± 10.7[b] | 5.1 ± 5.5[a] | 26.90 ± 0.25[a] |
| SUB | 28.1 ± 10.4[b] | 16.6 ± 7.8[a] | 4.1 ± 5.5[a] | 26.99 ± 0.33[a] |
| Hypo-responders (n = 13) | | | | |
| EGG | 21.8 ± 7.8[c] | 19.4 ± 10.5[ab] | 4.5 ± 5.5[a] | 26.58 ± 0.57[b] |
| SUB | 21.9 ± 8.0[c] | 19.1 ± 11.5[ab] | 4.6 ± 6.8[a] | 26.67 ± 0.62[b] |
| Men | | | | |
| Hyper-responders (n = 12) | | | | |
| EGG | 23.3 ± 8.4[c] | 19.6 ± 8.2[ab] | 16.6 ± 16.2[c] | 26.24 ± 0.58[b] |
| SUB | 20.0 ± 7.9[c] | 25.6 ± 5.6[b] | 9.9 ± 10.4[bc] | 26.40 ± 0.53[b] |
| Hypo-responders (n = 13) | | | | |
| EGG | 18.2 ± 8.8[d] | 20.5 ± 5.6[ab] | 6.8 ± 6.1[ab] | 26.75 ± 0.35[a] |
| SUB | 20.5 ± 9.0[c] | 20.5 ± 9.0[ab] | 7.8 ± 7.5[b] | 26.67 ± 0.52[b] |
| Sex effect | $P < .05$ | NS | $P < .01$ | $P < .01$ |
| Response effect | $P < .05$ | NS | NS | NS |
| Diet effect | NS | NS | $P < .05$ | NS |
| Interaction sex × response | NS | NS | NS | $P < .001$ |
| Interaction sex × diet | NS | NS | NS | NS |
| Interaction response × diet | $P < .001$ | $P < .05$ | $P < .001$ | NS |
| Interaction sex × diet × response | NS | $P < .01$ | $P < .05$ | NS |

NOTE. Values are presented as mean ± SD for the number of subjects indicated in parentheses. Values in the same column with different superscripts are significantly different as determined by 3-way ANOVA and LSD as post hoc test.

Abbreviations: EGG, egg; SUB, substitute; NS, not significant.

consumption, while hyporesponders experienced no change for either dietary period (Table 2). However, LDL oxidation was not affected by sex, diet, or response classification (Table 2). In contrast, plasma LCAT and CETP activities were significantly modulated by the response to dietary cholesterol. Following egg consumption, subjects classified as hyperresponders, re-

**Table 2. Plasma LDL Cholesterol, LDL Oxidation, and LCAT and CETP Activities of Hypo- and Hyper-responders during the EGG or SUB Periods**

| | LDL-C (mg/dL) | Apo B Lipoprotein Oxidation (TBARS of MDA/non-HDL protein) | LCAT (μmol/h · L plasma) | CETP (μmol/h · L plasma) |
|---|---|---|---|---|
| Women | | | | |
| Hyper-responders (n = 14) | | | | |
| EGG | 114.6 ± 32.0[a] | 19.8 ± 10.1 | 18.5 ± 7.9[a] | 23.8 ± 6.8[a] |
| SUB | 99.4 ± 30.5[b] | 16.4 ± 7.4 | 13.6 ± 6.9[a] | 23.0 ± 4.7[b] |
| Hypo-responders (n = 13) | | | | |
| EGG | 90.4 ± 25.4[b] | 15.3 ± 7.0 | 12.2 ± 6.1[b] | 21.5 ± 6.0[b] |
| SUB | 90.8 ± 28.7[b] | 15.7 ± 6.3 | 11.7 ± 5.7[b] | 19.7 ± 4.2[b] |
| Men | | | | |
| Hyper-responders (n = 12) | | | | |
| EGG | 118.6 ± 27.9[a] | 16.8 ± 8.5 | 18.5 ± 11.1[a] | 21.7 ± 6.8[a] |
| SUB | 91.9 ± 21.3[b] | 16.1 ± 4.1 | 15.9 ± 9.6[b] | 18.5 ± 4.4[b] |
| Hypo-responders (n = 13) | | | | |
| EGG | 87.5 ± 26.1[b] | 18.5 ± 5.2 | 13.5 ± 5.9[b] | 16.6 ± 3.0[b] |
| SUB | 89.7 ± 27.1[b] | 19.6 ± 6.0 | 14.9 ± 5.6[b] | 19.1 ± 6.6[b] |
| Sex effect | NS | NS | NS | $P < .01$ |
| Response effect | $P < .0001$ | NS | $P < .05$ | $P < .05$ |
| Diet effect | $P < .05$ | NS | NS | NS |
| Interaction sex × response | NS | NS | $P < .05$ | NS |
| Interaction sex × diet | NS | NS | NS | NS |
| Interaction response × diet | $P < .0001$ | NS | NS | NS |
| Interaction sex × diet × response | NS | NS | NS | $P < .05$ |

NOTE. Values are presented as mean ± SD for the number of subjects indicated in parentheses. Values in the same row with different superscripts are significantly different as determined by 3-way ANOVA and LSD as post hoc test.

**Table 3. Number of CE, FC, TG, and PL Molecules in LDL of Hypo- and Hyper-responders During the EGG or SUB Periods**

| | No. of Molecules/LDL | | | |
|---|---|---|---|---|
| | CE | FC | TG | PL |
| Women | | | | |
| Hyper-responders (n = 14) | | | | |
| EGG | 1,117 ± 239[ab] | 65 ± 20 | 99 ± 28[a] | 579 ± 95 |
| SUB | 1,036 ± 277[a] | 58 ± 15 | 96 ± 29[a] | 529 ± 95 |
| Hypo-responders (n = 13) | | | | |
| EGG | 1,020 ± 220[a] | 51 ± 18 | 181 ± 47[b] | 531 ± 82 |
| SUB | 1,013 ± 255[a] | 54 ± 20 | 178 ± 37[b] | 518 ± 102 |
| Men | | | | |
| Hyper-responders (n = 14) | | | | |
| EGG | 1,315 ± 204[c] | 69 ± 16 | 161 ± 32[b] | 537 ± 53 |
| SUB | 1,149 ± 248[b] | 65 ± 62 | 179 ± 46[b] | 519 ± 64 |
| Hypo-responders (n = 13) | | | | |
| EGG | 1,119 ± 240[b] | 77 ± 60 | 189 ± 39[bc] | 551 ± 95 |
| SUB | 1,213 ± 328[bc] | 73 ± 58 | 214 ± 74[c] | 586 ± 111 |
| Sex effect | $P < .01$ | NS | $P < .0001$ | NS |
| Response effect | NS | NS | $P < .0001$ | NS |
| Diet effect | NS | NS | NS | NS |
| Interaction sex × response | NS | NS | $P < .05$ | NS |
| Interaction sex × diet | NS | NS | $P < .05$ | NS |
| Interaction response × diet | $P < .05$ | NS | NS | NS |
| Interaction sex × diet × response | NS | NS | NS | NS |

NOTE. Values are presented as mean ± SD for the number of subjects indicated in parentheses. Values in the same row with different superscripts are significantly different as determined by 3-way ANOVA and LSD as post hoc test.

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TG, triglycerides; PL, phospholipids; NS, not significant.

gardless of gender, had higher activity of these components of reverse cholesterol transport ($P < .05$) (Table 2). In addition, women were found to have higher CETP activity than men (sex effect, $P < .05$).

*The Effect of Diet, Sex, and Response on the Composition of the LDL Particle*

Sex and diet modulated the composition of the LDL particle (Table 3). The number of CE and TG molecules in LDL was higher in men when compared with women ($P < .01$) (Table 3). In addition, hyporesponders had a higher number of TG molecules than was seen in the LDL particle of hyperresponders. In contrast, the number of PL and FC molecules was not affected by sex, diet, or response classification (Table 3).

## DISCUSSION

The link between genetics and LDL phenotype has been examined by various family studies.[32] Loci near the LDL receptor gene on chromosome 19p, the apo C-III gene on chromosome 11, and the CETP gene on chromosome 16 have been identified[33] because of their apparent association with LDL peak particle size. However, findings from twin studies contradict the possibility of complete genetic control over phenotype showing only a weak overall heritability of peak particle diameter in some cases.[34] These findings suggest that genetic predetermination of LDL phenotype may be modifiable by environmental factors such as age, sex, adiposity, macronutrient composition of the diet, hormones, and drugs.[35]

It has been shown that expression of the Pattern B subclass is greater in males older than 20 years compared with younger men or premenopausal women.[36,37] In fact, the frequency of the LDL pattern B phenotype in the general population is approximately 30% in men and 15% to 20% in postmenopausal women.[6] In the population from the current study, 70% of men were classified as having the B phenotype, while 37% of women were similarly identified. Therefore, as expected, women were found to have a greater predominance of the LDL-1 particle than men regardless of response classification. It has been suggested that higher visceral adipose accumulation in men may be the contributing factor to the sex difference seen in the determination of LDL size.[38] However, a comparison of LDL peak particle diameter between sex and response groups showed that a significant difference only existed between female and male hyperresponders with the latter having smaller particles. This difference did not exist between male and female hyporesponders. In fact, male hyporesponders had a significantly higher peak diameter than their female counterparts during the egg period, with no differences being observed following consumption of the placebo. These findings suggest that the influence of sex may not be driving the differences seen within these response groups. Therefore, perhaps the influence of diet on LDL phenotype was most prominent in this study.

Existing dietary prescriptions for the treatment and prevention of atherosclerosis and CHD are focused on reducing plasma LDL-C levels through the limitation of cholesterol and total fat intake with specific emphasis on restriction of saturated fat. Studies[39,40] that have examined the effects of such diets on lipoprotein concentrations have shown a wide variation among individuals with some concluding that a low-fat/high-carbohydrate diet may actually increase risk by causing a general increase in plasma concentrations of TG[41] and decreased HDL-C. This response has been shown to be even 2-fold

greater in persons with the pattern B phenotype.[42] It has been suggested that these concomitant fluctuations would negate any positive effects of the lowered LDL-C achieved by the modification in macronutrient consumption. An examination of postprandial lipoprotein metabolism suggests that the pattern B subclass is associated with an overall decrease in the clearance rate of intravenous fat[43] and an increase in the occurrence of lipemia.[44] Furthermore, with regard to LDL subclass, pattern A individuals have also been found to have less of a reduction in LDL-C in response to a low-fat diet than those who have the pattern B phenotype.[45,46] A shift to the pattern B subclass has also been detected in individuals initially classified as pattern A when a low-fat/high-carbohydrate diet was consumed.[47]

Because 1 large egg contains approximately 5.01 g total lipid and 213 mg cholesterol, both response groups consumed significantly more of these 2 components during the egg as compared with the placebo period. Furthermore, the consumption of fat during the egg period was consistent with a typical "western" diet (approximately 31% of energy from total fat), while the placebo period was significantly lower in fat (approximately 26% of energy from fat) and higher in carbohydrate. Therefore, due to the macronutrient composition of the diet during the egg period, a predominance of LDL-1 particles would be expected and was found in both male and female hyperresponders. However, male and female hyporesponders did not experience the same increase in LDL-1 concentrations following egg consumption.

Increased intake of dietary fat has also been associated with increased activities of lipoprotein lipase (LPL) and hepatic lipase (HL) in humans.[48] LPL's role in metabolism is to hydrolyze the TG components in chylomicrons and VLDL and promote the cellular uptake of these particles. HL functions to hydrolyze the TG and phospholipids (PL) contained in LDL, which results in the production of a smaller more dense particle.[49] The transfer of CE from HDL to apo B-containing lipoproteins in exchange for TG is mediated by CETP. Generally, increased CETP activity is regarded as proatherogenic. However, if an increase in CETP is not related to a decrease in HDL-C, as we saw in this study,[16,17] this protein appears to function in an antiatherogenic manner by enhancing CE enrichment of LDL particles that can be taken up and metabolized by the liver.[50] Increased CETP activity may also inhibit HL-mediated modification of the apo B-containing lipoproteins because TG-rich, not CE-rich, LDL particles are the preferred substrate for this lipase. Decreased HL activity is associated with elevated large more buoyant LDL particles. Therefore, the predominance of LDL-1 particles that was seen in hyperresponders, regardless of gender, may be due to the increased activity of CETP during the egg period. Furthermore, the finding that CETP activity was greater in women when compared with men provides another explanation as to why the female participants were found to have higher concentrations of the LDL-1 particle. In contrast, previous research has identified the presence of a negative correlation between LDL size and CETP activity.[51] Additional evidence obtained from an in vitro study showed that treatment of cells with endogenous plasma CETP and LCAT and exogenous LPL resulted in the production of smaller more dense LDL particles.[52] However, this lipoprotein modification became more pronounced as the TG concentration increased in the plasma. In the current study, participants had normal TG concentrations at baseline, and no changes were seen following dietary treatment nor were they different between response or gender groups.[16,17] It has been suggested that CETP only functions in a negative capacity with regard to LDL size determination if hypertriglyceridemia is present.[50]

Plasma LDL-C concentration is widely used as a diagnostic tool for the prediction of atherosclerosis. However, plasma levels of individual lipoproteins may have restricted prognostic importance.[5] Approximately 30%[53,54] of patients with diagnosed premature coronary artery disease (CAD) have plasma lipoprotein values that are within a range that is considered normal by NCEP standards.[55] In contrast, LDL subclass determination does appear to be a good predictor of atherosclerosis progression.[56,57] In fact, the Quebec Cardiovascular Cohort study,[58] which examined 2,034 men, found that a predominance of LDL particles with a peak diameter of $<$25.5 nm was positively associated with an increased risk for ischemic heart disease (relative risk = 4.6, $P < .001$). In addition, this association was found to be independent of variations in plasma concentrations of LDL-C, HDL-C, TC, TG, and Lp(a).

If the traditional method for assessment of risk were to be utilized, the hyperresponders in this study would be expected to have an increase in risk following egg intake due to the resulting elevations in LDL-C. However, this population was found to have a predominance of the larger LDL-1 subclass and the concentration was highest following egg consumption. Furthermore, LDL oxidation was not affected by sex, diet, or response classification. These findings clearly illustrate the discrepancy that exists between the utilization of LDL-C levels and lipoprotein particle size as predictors of disease risk. In conclusion, the results of this study clearly indicate that egg intake by a healthy population of men and premenopausal women does not have negative health implications with regard to LDL atherogenicity.

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