Original Research

Pre-Menopausal Women, Classified as Hypo- or Hyper-Responders, do not Alter their LDL/HDL Ratio Following a High Dietary Cholesterol Challenge

Kristin L. Herron, MS, Sonia Vega-Lopez, PhD, Karin Conde, PhD, Tripurasundari Ramjiganesh, PhD, Suheeta Roy, PhD, Neil S. Shachter, MD, and Maria Luz Fernandez, PhD

Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut (K.L.H., S.V.-L., T.R., S.R., M.L.F.) and Columbia University, New York, New York (K.C., N.S.S.)

Key words: dietary cholesterol, metabolic response, pre-menopausal women, reverse cholesterol transport, LCAT, CETP, Apo B, Apo C-III

Background: Cholesterol is the dietary component that has elicited the most public interest in conjunction with coronary heart disease. However, the impact of excess dietary cholesterol intake on plasma cholesterol levels cannot be accurately predicted; therefore, its role in disease progression is not straightforward. Individual response variation can be due to factors such as ethnicity, hormonal status, obesity and genetic predisposition.

Objective: The purpose of this study was to evaluate the differences that occur within the plasma compartment of normolipidemic pre-menopausal women, classified based on their response to a high dietary cholesterol challenge.

Design: We recruited 51 pre-menopausal women (29 Caucasian and 22 of Hispanic origin) aged 18 to 49 years with initial plasma cholesterol concentrations ranging from 3.62 to 5.17 mmol/L. Using a cross-over research design, women were randomly allocated to an egg (640 mg additional dietary cholesterol per day) or placebo group (0 mg additional dietary cholesterol per day) initially, and the two 30 day periods were separated by a three-week washout.

Results: An initial evaluation of the ethnicity effects revealed elevations in both plasma LDL-C ($p < 0.0001$) and HDL-C ($p < 0.001$) concentrations in both Hispanics and Caucasians during the high dietary cholesterol period. However, these increases were not accompanied by a change in the LDL/HDL ratio. Subjects were then classified as hypo- (< 0.05 mmol/L increase in total plasma cholesterol per each additional 100 mg of dietary cholesterol consumed per day) or hyper-responders (≥ 0.06 mmol/L increase in total blood cholesterol per each additional 100 mg of dietary cholesterol consumed per day), based on their reaction to the additional dietary cholesterol provided. Hypo-responders did not experience an increase in LDL-C or HDL-C during the egg period, while both lipoproteins were elevated in hyper-responders. However, the LDL/HDL ratio, an important parameter of coronary heart disease risk, was maintained for all subjects during the egg period independent of response. Furthermore, hyper-responders had higher concentrations of apo C-III ($p < 0.001$), apo B ($p < 0.001$) and cholesterol ester transfer protein (CETP) ($p < 0.05$) during this period.

Conclusion: These data revealed that excess dietary cholesterol does not increase the risk of developing an atherogenic lipoprotein profile in pre-menopausal women, regardless of their response classification. Although the addition of 640 mg of cholesterol to the diet did result in an increase in plasma cholesterol in hyper-responders, the LDL/HDL ratio was maintained. This result, accompanied by increases in CETP activity, leads to the speculation that hyper-responders may process the excess cholesterol in the plasma compartment through an enhancement of the reverse cholesterol transport pathway. With this mechanism identified, further measurement of additional parameters is needed to verify this conclusion.

Address reprint requests to: Kristin L. Herron, MS, Department of Nutritional Sciences, University of Connecticut, 3624 Horsebarn Road Extension, Storrs, CT 06269. E-mail: kristin.herron@uconn.edu.

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INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States [1]. Risk factors such as obesity, hypertension, diabetes, elevated serum cholesterol and related disorders of serum lipoproteins promote the development of atherosclerosis and CHD. The production, remodeling and removal of lipoproteins are dynamic processes that are mediated by various factors such as enzymes, transfer proteins and apoproteins. Genetic composition and dietary intake influence these factors and the mechanisms by which they affect lipoprotein metabolism.

It is difficult to predict accurately the impact of dietary cholesterol on plasma cholesterol levels because individuals do not experience a uniform response. Individual variation can be due to factors such as ethnicity, hormonal status, obesity, lipoprotein disorders, genetic predisposition and basal plasma lipid levels [2,3]. Data gathered from hundreds of cholesterol-feeding studies conducted over the last 50 years have provided the conclusion that a modest increase in total plasma cholesterol of 0.05–0.06 mmol/L can be predicted in response to a 100 mg/day increase in dietary cholesterol [4]. Therefore, hypo-responders can be classified as those who experience an increase in total cholesterol < 0.05 mmol/L/100 mg of additional dietary cholesterol consumed per day, and hyper-responders are those who have an increase ≥ 0.06 mmol/L. Although this classification is precise, the mechanisms that govern this increase in the plasma compartment of hyper-responders remain rather ambiguous.

Hypo-responders may have the ability to maintain cholesterol homeostasis by decreasing synthesis [5], absorption [6] or increasing biliary excretion [7,8]. Evidence has been presented that supports the conclusion that hypo-responders decrease absorption in response to the ingestion of large amounts of dietary cholesterol. Through the provision of an oral dose of [^{14}C] cholesterol, McNamara *et al.* [9] determined that absorption could be reduced by approximately 6% as the cholesterol content of the diet was increased from 240 mg/day to 840 mg/day. An additional study by Ostlund *et al.* [10] showed a similar decrease in intestinal absorption in response to acute doses of dietary cholesterol within a range of 26 mg to 421 mg. The mechanisms responsible for this decreased absorption in hypo-responders are unclear; however, dietary factors such as intake of phytosterols [11] and genetic factors such as the apo E polymorphism [12] may affect this response. In contrast, hyper-responders may either absorb more dietary cholesterol or may be unable to suppress effectively endogenous synthesis as compensatory mechanisms in response to excess intake. This could explain the observed increases in the amount of circulating cholesterol that must be handled by this population.

The objectives of this study were to further clarify the differences that occur within the plasma compartment following a dietary cholesterol challenge in pre-menopausal women classified as hypo- or hyper-responders. The second objective was to evaluate how hyper-responders process the excess cholesterol in the plasma compartment. Based on our findings that hyper-responders experienced significant increases in LDL and HDL cholesterol and in cholesterol ester transfer protein (CETP) activity after consumption of excess dietary cholesterol, we hypothesized that this group may increase reverse cholesterol transport as a mechanism by which increased circulating cholesterol levels are handled.

SUBJECTS AND METHODS

Materials

Liquid whole eggs and cholesterol/fat free eggs (placebo) were purchased from Better Brands Inc. (Windsor, CT). Enzymatic cholesterol and triglyceride kits were from Boehringer-Mannheim (Indianapolis, IN). Apo C-III and apo E kits were from Wako Pure Chemical (Osaka, Japan). Apo B kits, EDTA, aprotinin, sodium azide and phenyl methyl sulfonyl fluoride (PMSF) were obtained from Sigma Chemical (St. Louis, MO).

Subjects

Hispanic and Caucasian pre-menopausal women, between the ages of 18 to 49 years, with plasma cholesterol levels within the range of 3.62–5.17 mmol/L, were recruited from the University community. Hypertriglyceridemic, hypertensive and diabetic subjects were excluded from the study, along with those receiving lipid-lowering drugs or having a plasma cholesterol level > 5.69 mmol/L. For this study, a total of 29 Caucasian and 22 women of Hispanic origin completed the study. The majority of the Hispanic participants were of Puerto Rican decent; however, Mexicans and South Americans were also represented in the sample. Initial characteristics of the subjects are presented in Table 1.

Table 1. Baseline Characteristics of Hispanic and Caucasian Women[1]

Parameter	Caucasians (n = 29)	Hispanics (n = 22)	*p*-value
Age (years)	31.1 ± 9.2	27.9 ± 7.2	N.S.
No. of smokers	1	2	—
Physical Activity (hrs/wk)	5.0 ± 3.2[a]	3.0 ± 2.9[b]	<0.05
BMI (kg/m^2)	23.6 ± 3.2	24.6 ± 6.7	N.S.
Systolic blood pressure (mm Hg)	117.4 ± 8.9	114.3 ± 8.1	N.S.
Diastolic blood pressure (mm Hg)	74.2 ± 6.2	74.5 ± 8.0	N.S.
Total cholesterol (mmol/L)	4.62 ± 0.79	4.39 ± 0.70	N.S.
LDL cholesterol (mmol/L)	2.56 ± 0.74	2.36 ± 0.82	N.S.
HDL cholesterol (mmol/L)	1.58 ± 0.30	1.48 ± 0.32	N.S.
Triglycerides (mmol/L)	0.92 ± 0.41	1.02 ± 0.54	N.S.

[1] Data are presented as mean ± SD. Values in the same row with different superscript are significantly different as determined by paired *t* test. N.S. = non-significant.

Experimental Protocol

The experimental protocol was approved by the University of Connecticut Institutional Review Board, and written informed consent was obtained from each subject. The study utilized a randomized cross-over design, with subjects initially assigned to an egg or placebo group for 30 days, followed by a three-week washout period, after which the second dietary period began. Subjects assigned to the egg group were expected to consume the equivalent of three eggs per day (approximately 640 mg dietary cholesterol). In contrast, the placebo group consumed an identical weight of egg substitutes (0 mg dietary cholesterol). Daily amounts were provided in individual containers, and subjects were asked to return any uneaten portion at the end of the week.

Subjects were also expected to adhere to the National Cholesterol Education Program (NCEP) Step I diet, and detailed dietary instructions were provided. The NCEP Step I diet recommends that no more than 30% of total calories come from fat and no more of 10% of total calories be from saturated fat. In addition, subjects were instructed to consume less than 300 mg of dietary cholesterol per day. To ensure compliance with the dietary guidelines, subjects completed seven 24-hour dietary records during each treatment period including two weekend days. Nutrient intake was calculated using the Nutrition Data System for Research (NDS-R) software Version 4.0, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 28. Subjects also completed a food frequency questionnaire at the beginning of the study to illustrate regular nutrient intake.

Two fasting (12 hours) blood samples were obtained for each subject and placed into tubes containing 0.15% EDTA to determine baseline plasma lipids. Plasma was separated by centrifugation at 1,500 g for 20 minutes at 4°C and placed into tubes containing phenyl methyl sulphonyl fluoride (0.05%), sodium azide (0.01%) and aprotonin (0.01%). Two additional blood samples were collected at the end of each diet treatment and washout period. The additional variables of weight, blood pressure, level of activity, smoking and alcohol intake were 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 and Apolipoproteins

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 total cholesterol and triglyceride assays. Coefficients of variance assessed by the Standardization program during the study period were 0.76–1.42 for total cholesterol, 1.71–2.72 for HDL-C and 1.64–2.47 for triglycerides.

The effect of dietary cholesterol on triglycerides, total, LDL and HDL cholesterol levels and the LDL/HDL ratio was examined. Total cholesterol was determined by enzymatic methods using Boehringer Mannheim standards and kits [13]. HDL cholesterol was measured in the supernatant after precipitation of apo B-containing lipoproteins [14], and LDL cholesterol was determined using the Friedewald equation [15]. Triglycerides were determined using Boehringer-Mannheim kits, which adjust for free glycerol. Means of the two blood draws were used to assess differences between treatment periods. Apolipoprotein B concentrations were determined using an immunoturbidimetric method, and turbidity was measured in a microplate spectrophotomer at 340 nm [16]. Apo C-III [17] and apo E [18] were measured with a Hitachi Autoanalyzer 740 utilizing kits from Wako.

Classification of Hyper- and Hypo-Responders

Generally, a modest increase in total cholesterol of 0.05–0.06 mmol/L can be predicted in response to a 100 mg/day increase in dietary cholesterol [4]. Therefore, for the purpose of this study, subjects who experienced an increase in total cholesterol $\geq$0.06 mmol/L for each additional 100 mg of dietary cholesterol were considered hyper-responders. Because the subjects were fed an additional 640 mg/day of dietary cholesterol, those who experienced an increase in total cholesterol of 0.38 mmol/L or greater would be considered hyper-responders. The remaining subjects who experienced fluctuations of $<$0.32 mmol/L (an increase in total cholesterol of $<$0.05 mmol/L for each additional 100 mg of dietary cholesterol consumed) or had no change in total cholesterol were identified as hypo-responders.

Plasma CETP and LCAT Determinations

CETP activity was determined using plasma from all human subjects according to Ogawa & Fielding [19], which measures the mass transfer of cholesterol ester between HDL and apo B containing-lipoproteins. Thus, physiological CETP activity was found through an analysis of the decrease in HDL cholesterol ester mass between 0 and 6 hours, without 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 [20]. 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 carried out concurrently with measurements of CETP. Both of these methods have been standardized in our laboratory.

Data Analysis

Student's *t* test was used to analyze baseline characteristics of Hispanic and Caucasian pre-menopausal women. Repeated-measures ANOVA was used to determine how ethnicity affects

the response to dietary cholesterol on plasma lipids. Because subjects were separated into hyper- and hypo-responders after data collection, a paired *t* test was used to evaluate plasma lipids, apo-proteins, CETP and LCAT activities during the egg and placebo periods. A paired *t* test was also used to determine dietary consumption of macronutrients, dietary cholesterol, alcohol and dietary fiber.

RESULTS

Baseline physical characteristics and fasting lipid levels for both ethnic groups are presented in Table 1. There were no significant differences with regard to age, body mass index (BMI), systolic and diastolic blood pressure or plasma lipids between the two groups. The greater number of hours of physical activity per week reported by Caucasians was the only observed difference between Hispanic and Caucasian women.

Subject compliance, with regard to the egg and placebo consumption, was assessed weekly by recording any returned portion. There were no significant differences in intake related to dietary period or ethnicity. Caucasians consumed 98.0 ± 6.0 and 98.7 ± 3.3% of the daily portion of eggs and placebo respectively, while Hispanics consumed 99.3 ± 1.0% of the provided eggs and 98.7 ± 2.7% of the placebo.

Ethnicity did not play a role in the plasma lipid response to dietary cholesterol intake; however, a dietary effect was evident (Table 2). Plasma TC, LDL-C and HDL-C were significantly higher ($p < 0.0001$) during the egg consumption period for both ethnic groups when compared to placebo. In contrast, the TC/HDL and the LDL/HDL ratios were maintained during both dietary periods (data not shown). Because differences between ethnic groups were not apparent, we proceeded with the classification of subjects into hyper- and hypo-responders as described in the Methods section.

Table 2. Plasma Total Cholesterol (TC), LDL and HDL-C of Hispanic and Caucasian Women during the Egg and Placebo Dietary Periods[1]

	Cholesterol (mmmol/L)		
	TC	LDL-C	HDL-C
Eggs			
Caucasians	4.76 ± 0.83	2.67 ± 0.77	1.70 ± 0.35
Hispanic	4.64 ± 0.75	2.56 ± 0.72	1.58 ± 0.43
Placebo			
Caucasians	4.41 ± 0.83	2.38 ± 0.65	1.60 ± 0.35
Hispanic	4.41 ± 0.70	2.47 ± 0.63	1.52 ± 0.43
Repeated Measures ANOVA			
Diet Effect	$p < 0.0001$	$p < 0.001$	$p < 0.001$
Ethnicity effect	N.S.	N.S.	N.S.
Interaction	N.S.	N.S.	N.S.

[1] Values are presented as mean ± SD for N = 29 Caucasians and 22 Hispanics. N.S. = non-significant.

A careful analysis of our data indicated that the subjects who participated in the study could be classified into one of two major groups: hypo- or hyper-responders. This classification demonstrated that, while some individuals have a slight or no change in plasma cholesterol following a high dietary cholesterol challenge, others exhibit a higher response. All of our subjects identified as hypo-responders experienced increases in plasma cholesterol below what is considered to be a normal response to the provided dietary cholesterol challenge [4]. In contrast, hyper-responders had increases in plasma cholesterol that were ≥0.06 mmol/L/100 mg additional dietary cholesterol, which is equivalent to an increase of at least 0.38 mmol/L when based on the amount of additional cholesterol consumed in this study. The use of this criterion resulted in the classification of 20 hyper-responders and 31 hypo-responders. Therefore, 60% of the study population were considered hypo-responders.

BMI, body weight, blood pressure (both systolic and diastolic) and hours of physical activity were evaluated during the egg and placebo periods to account for the effects of these parameters on plasma lipids. As indicated in Table 3, both hypo- and hyper-responders maintained their body weights as assessed by BMI during the entire length of the study. In addition, the systolic and diastolic blood pressures and the hours of physical activity reported per week were not different between dietary periods, indicating that these parameters did not affect the response to dietary cholesterol (Table 3).

Dietary intake was evaluated for both hyper- and hypo-responders to determine whether the response to dietary cholesterol could be related to differences in macronutrient intake. As shown in Table 4, an analysis of the two provided seven-day dietary food records showed that subjects complied with the study requirement to follow the NCEP Step I diet during both the egg and placebo periods. The analysis revealed that both hyper- and hypo-responders had significant increases in the percentage of energy derived from total, saturated and monounsaturated fat during the egg compared to the placebo period. However, consumption of polyunsaturated fat was higher only in hyper-responders during the egg period (Table 4). As expected, a very significant increase in dietary cholesterol was observed for hyper- and hypo-responders during the egg period ($p < 0.0001$).

Other differences in dietary intake were observed between periods. For example, during the placebo period both groups consumed 56% more energy from alcohol on average than they did during egg period ($p < 0.01$). Soluble fiber intake was also greater during placebo consumption, when compared to the egg period, for both hyper- and hypo-responders ($p < 0.001$). In addition, during the placebo period the percentage of energy derived from carbohydrates was higher for hypo-responders ($p < 0.001$), while the percentage of energy derived from protein was higher for hyper-responders ($p < 0.01$). Hyper-responders consumed 46.9 ± 8.8% and 50.0 ± 9.9% while hypo-responders consumed 49.1 ± 8.3% and 53.1 ± 7.1% of

Table 3. Body Mass Index, Kg, Blood Pressure and Hours of Physical Activity of Hyper and Hypo-Responders during the Egg and Placebo Period[1]

	BMI (kg/m2)	Weight (Kg)	SBP (mm Hg)	DBP (mm Hg)	Physical Activity (h/wk)
Hyper-Responders					
Egg	24.61 ± 4.83	65.2 ± 14.9	114.4 ± 8.3	72.5 ± 8.2	4.1 ± 3.1
Placebo	24.58 ± 5.20	65.1 ± 15.6	115.1 ± 8.7	72.6 ± 7.8	4.1 ± 3.1
Hypo-Responders					
Egg	23.71 ± 5.03	62.6 ± 13.9	117.1 ± 8.5	73.1 ± 7.1	4.4 ± 3.2
Placebo	23.57 ± 4.95	62.4 ± 14.0	116.4 ± 10.1	76.5 ± 8.3	4.4 ± 3.2

[1] Values represent mean ± SD for N = 31 hypo- and 20 hyper-responders. None of these parameters was significantly different between dietary periods as determined by paired *t* test.

Table 4. Percent (%) of Energy from Total Fat, Saturated (SAT), Monounsaturated (MONO) and Polyunsaturated (PUFA) Fat of Hyper- and Hypo-Responders during the Egg and Placebo Periods[1]

	% Energy Total FAT	% Energy SAT	% Energy MONO	% Energy PUFA	Dietary Cholesterol (mg/d)
Hyper-Responders					
Egg	34.3 ± 6.0	11.8 ± 3.0	12.8 ± 3.0	6.4 ± 1.6	748.9 ± 60.5
Placebo	29.0 ± 6.9	10.5 ± 3.3	11.3 ± 2.5	5.6 ± 2.4	160.6 ± 80.2
Paired *t* test	$p < 0.01$	$p < 0.05$	$p < 0.001$	$p < 0.05$	$p < 0.0001$
Hypo-Responders					
Egg	33.1 ± 6.2	11.3 ± 2.5	12.4 ± 2.7	5.7 ± 1.3	775.3 ± 69.0
Placebo	27.3 ± 7.1	9.5 ± 2.9	10.3 ± 3.1	5.5 ± 1.8	146.5 ± 80.2
Paired *t* test	$p < 0.01$	$p < 0.01$	$p < 0.01$	N.S.	$p < 0.0001$

[1] Values are expressed as mean ± S.D. for N = 20 Hyper- and N = 31 hypo-responders. N.S. = non-significant.

their energy from carbohydrates during the egg and placebo periods, respectively. The percentages of energy derived from protein were 17.9 ± 4.1% (egg) and 20.0 ± 4.6% (placebo) for hyper-responders, while hypo-responders consumed 18.4 ± 4.4% (egg) and 19.4 ± 4.0% (placebo).

The response classification also revealed a considerable effect on lipid levels. As shown in Table 5, hyper-responders experienced significant increases in LDL-C ($p < 0.0001$) and HDL-C ($p < 0.001$) during the egg period. In contrast, hypo-responders maintained their LDL-C and HDL-C concentrations during both periods. Furthermore, no effect of egg consumption was seen on the LDL/HDL ratio for either group. In addition, neither diet nor level of response had any effect on plasma triglycerides (Table 5). Fig. 1 further illustrates the two responses to dietary cholesterol by presenting the changes in total (upper panel) and HDL cholesterol (lower panel) that were experienced by each subject during the egg period when compared to the placebo.

Several important differences between hypo- and hyper-responders with regard to intravascular lipoprotein processing were also found (Table 6). Apo B ($p < 0.001$), Apo C-III ($p < 0.001$) and CETP activity ($p < 0.05$) were higher in hyper-responders during the egg period while no changes were observed in hypo-responders. Apo E concentrations were not modified by diet or by the response to dietary cholesterol (Table 6).

Table 5. Plasma Lipids and Lipoprotein Ratios of Hyper- and Hypo-Responders during the Egg and Placebo Periods[1]

	LDL-C	HDL-C (mmol/L)	Triglycerides	LDL/HDL
Hyper-Responders				
Egg	3.02 ± 0.72	1.76 ± 0.40	0.91 ± 0.44	1.80 ± 0.65
Placebo	2.50 ± 0.70	1.57 ± 0.27	0.89 ± 0.41	1.63 ± 0.52
Paired *t* test	$p < 0.0001$	$p < 0.001$	N.S.	N.S.
Hypo-Responders				
Egg	2.36 ± 0.64	1.56 ± 0.34[b]	0.90 ± 0.46	1.62 ± 0.74
Placebo	2.40 ± 0.61	1.53 ± 0.31[b]	0.93 ± 0.43	1.63 ± 0.61
Paired *t* test	N.S.	N.S.	N.S.	N.S.

[1] Values are expressed as mean ± S.D. for N = 20 Hyper- and N = 31 Hypo-responders. N.S. = non-significant.

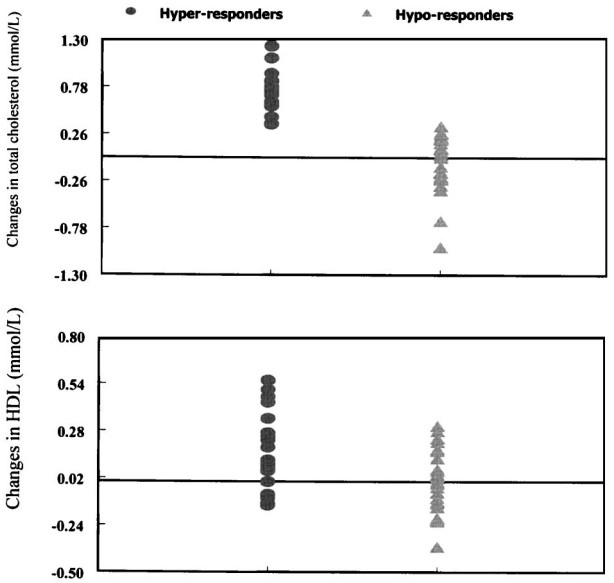

Fig. 1. Changes in total (upper panel) and HDL-C (lower panel) in the egg compared to the placebo period for hyper- (●) (n = 20) and hypo-responders (▲) (n = 31).

DISCUSSION

We expected that ethnicity would play a significant role in the plasma lipid response to dietary cholesterol intake. Although differences between ethnic groups were not seen, the further classification of subjects as hyper- or hypo-responders clearly indicated the presence of metabolic differences in the intravascular handling of excess circulating cholesterol. Therefore, it was important to determine that the plasma lipid changes experienced by hyper-responders were due to the high cholesterol challenge and not to other dietary factors that are known to be influential.

Diet and Plasma Cholesterol

The dietary intake of both hyper- and hypo-responders varied significantly between treatment periods. The increases seen in energy from total fat, MONO, PUFA and dietary cholesterol were expected and can be attributed to the eggs. Whole eggs provide 75 kcal, 5.01 g total lipid (1.5 g from saturated fat), 6.25 g protein and 0.61 g of carbohydrates [21]. In comparison, although the placebo is void of fat and cholesterol, the egg substitute that was used in this study does contain the same quality and amount of protein. During the egg period, subjects consumed a lower percentage of kcal from carbohydrates and less soluble fiber. One explanation for this occurrence may be that, because the placebo was less energy dense due to the lack of fat, subjects increased their intake of grain products to compensate for the caloric reduction.

In addition, alcohol consumption was greater during the placebo period independent of response. Evidence indicates that dietary composition affects alcohol consumption. A high carbohydrate/low protein diet has been shown to suppress alcohol intake [22]. In contrast, when fed a high fat/low carbohydrate diet, hamsters significantly lowered their consumption of alcohol [23]. Moderate alcohol consumption has been found to increase HDL-C; this may explain its protective effect against coronary heart disease [24,25]. Because both groups were consuming less alcohol during the egg period, the observed increases in HDL-C must be attributed to the higher cholesterol intake.

Subjects also reported elevated intakes of saturated, monounsaturated and polyunsaturated fatty acids during the egg period. Saturated fat intake has been shown to produce an elevation in plasma LDL-C levels [26]. A fluctuation in LDL-C of 0.0465 mmol/L can be expected for every 1% change in saturated fat intake with relation to the % of total energy consumed [2]. In contrast, diets that replace saturated fat with MONA and PUFA result in a decrease in plasma cholesterol concentrations [27]. By increasing PUFA intake by 1% of total energy intake, a 0.0129 mmol/L decrease in LDL-C can be predicted [2]. Therefore, the increased combined consumption of these three fatty acids during the egg period by both groups may have proved to counterbalance the effects anticipated from either component alone. In addition, hypo-responders did not increase their intake of PUFA during the egg period; this again indicates that the observed responses can be attributed to the higher cholesterol intake. However, to further account for possible changes due to fat consumption, the following equations were used to predict changes in total cholesterol and LDL-C:

$$\Delta\text{Serum Total Cholesterol } (\mu\text{mol/L}) = 49.599 \cdot \Delta\text{SFA} - 23.274 \cdot \Delta\text{PUFA} + 0.5741 \cdot \Delta\text{dietary cholesterol}$$

$$\Delta\text{LDL-C } (\mu\text{mol/L}) = 46.755 \cdot \Delta\text{SFA} - 12.801 \cdot \Delta\text{PUFA}$$

Using these equations, hyper-responders could be expected to experience an increase of 0.383 μmol/L in total cholesterol, while a 0.632 μmol/L increase would be predicted for hypo-responders based on the changes in fat intake between the two dietary periods. In addition, changes in LDL-C of 0.050 μmol/L (1.95 mg/dL) and 0.083 μmol/L (3.21 mg/dL) could be expected for hyper-responders and hypo-responders respectively. Due to the insignificant contribution of fat in the eggs to the measured plasma lipid concentrations, we can conclude that changes were identified as a result of the excess dietary cholesterol provided.

Because the results of this study confidently show that the increase in cholesterol found in the plasma compartment of hyper-responders can be attributed to the high dietary cholesterol consumption, we can speculate as to what mechanisms may be responsible for this occurrence. The absence of an increase in plasma cholesterol in hypo-responders may be explained by their ability to maintain cholesterol homeostasis

Table 6. Plasma Apoproteins and LCAT and CETP Activities in Hyper- and Hypo-Responders during the Egg and Placebo Periods[1]

	Apoproteins (mg/dL)			Activity (nmol/h.mL plasma)	
	Apo B	Apo E	Apo C-III	LCAT	CETP
Hyper-Responders					
Egg	78.6 ± 24.0	3.4 ± 0.7	13.9 ± 2.6	23.5 ± 6.7	19.1 ± 7.3
Placebo	67.5 ± 22.1	3.2 ± 0.6	12.1 ± 2.5	22.9 ± 4.6	14.9 ± 8.4
Paired *t* test	$p < 0.001$	N.S.	$p < 0.001$	N.S.	$p < 0.05$
Hypo-Responders					
Egg	67.8 ± 16.8	3.3 ± 1.0	12.7 ± 2.4	20.2 ± 5.5	13.0 ± 6.2
Placebo	68.5 ± 19.0	3.4 ± 1.0	12.5 ± 3.0	20.0 ± 4.2	13.2 ± 6.4
Paired *t* test	N.S.	N.S.	N.S.	N.S.	N.S.

[1] Values are expressed as mean ± S.D. for N = 20 hyper and N = 31 hypo-responders. N.S. = non-significant.

by decreasing the absorption of dietary cholesterol or suppressing endogenous synthesis. In contrast, we speculate the hyper-responders are unique in that absorption and synthesis of cholesterol may not be affected by increased dietary intake; therefore, the mechanism by which the excess circulating cholesterol is processed may be through an enhancement of the reverse cholesterol transport pathway.

Differences in Plasma Lipoproteins between Hyper- and Hypo-Responders

An inverse relationship between HDL-C and CHD clearly exists [28]. There are several mechanisms that may explain this association. The HDL particle has the ability to collect cholesterol from the arterial wall; this ability protects against lesion development. In addition, low HDL-C is considered a marker for the presence of other CHD risk factors such as increased triglycerides and dense LDL [28]. Furthermore, HDL is considered to be anti-atherogenic because of its central role in reverse cholesterol transport.

Peripheral cells cannot degrade sterols; therefore, they rely on the transfer of neutral lipids to lipoproteins. Excess free cholesterol, collected by HDL, must be esterified immediately by LCAT to preserve the hydrophilic nature of the particle and to maintain the concentration gradient for further intake [29]. Because the role of LCAT is essential to this transport process, the enzyme is considered anti-atherogenic. In studies with transgenic rabbits, [30,31] overexpression of LCAT produced an increase in plasma HDL-C and a reduction in atherosclerotic plaque. However, the cholesteryl ester (CE) in the HDL particle has an alternate fate as well. CE can be transferred to the apo B containing lipoproteins in exchange for triglycerides. This transfer is mediated by CETP. Because increased CETP activity promotes this enrichment of circulating apo B containing lipoproteins with CE, and is usually associated with a decrease in HDL-C, it is regarded as pro-atherogenic [32]. However, if an increase in CETP is not related to a decrease in HDL-C, the protein appears to function in an anti-atherogenic manner by enhancing reverse cholesterol transport [33] through enriched LDL particles that can be taken up by the liver, where the cholesterol ester components are metabolized. This process represents an indirect pathway of reverse cholesterol transport that may also be enhanced, in some individuals, in response to increased dietary cholesterol intake. The findings of this study indicate that hyper-responders have distinctive mechanisms in place to handle fluctuations in cholesterol levels that occur within the plasma compartment as a result of excess dietary cholesterol consumption. During the high cholesterol period, hypo-responders did not raise their LDL or HDL cholesterol when compared to the placebo period. In contrast, the hyper-responders experienced significant increases in HDL-C, LDL-C and CETP. The higher concentrations and levels of activity of these parameters suggest that hyper-responders enhanced reverse cholesterol transport to mobilize the excess plasma cholesterol to the liver, the major site of cholesterol elimination from the body.

Hyper-responders also had an increase in apoproteins B and C-III during the egg period. The increase in Apo B can be attributed to the increase in LDL seen in this population due to the fact that it is the predominant apoprotein found on the particle. In addition, the observed elevated levels of apo C-III could be associated with an inhibition of lipoprotein lipase [34]; however, there was no change in plasma triglycerides during the egg period. The increase in apo C-III is more likely an association of this apoprotein with HDL due to the increased content of cholesterol in this lipoprotein during the high cholesterol period.

The results of this study suggest that hyper-responders may enhance the reverse cholesterol transport process in response to the excess dietary cholesterol consumed. The complimentary increases in HDL-C and CETP activity provide evidence that hyper-responders may cope with the cholesterol surplus in the plasma compartment by stimulating its transport to the liver through an indirect route via the LDL particle. Although increases in LDL are generally considered pro-atherogenic, hyper-responders did not experience a change in the LDL/HDL ratio, which is an important predictor of coronary heart disease

risk. Other studies [35–36] have also found egg consumption to be neutral in a similar manner.

Cholesterol has been the dietary component that has elicited the most public interest in conjunction with disease prevention. In an attempt to reduce the risk of developing CHD, many people have adopted a strategy of dietary modification, which eliminated high cholesterol products such as eggs. However, considering that the link between dietary cholesterol and disease progression has not been established, despite extensive research, this restriction seems avoidable.

In this study, we have shown that dietary cholesterol restriction is unwarranted for certain populations. The results indicate that pre-menopausal women with initial plasma cholesterol concentrations that place them at a low risk for CHD do not experience the development of an atherogenic lipoprotein profile following the consumption of a high dietary cholesterol challenge, regardless of their response classification. All of the women in our study had plasma cholesterol concentrations in the range of 3.62 to 5.69 mmol/L. Furthermore, 90% of these subjects also had an LDL-C level lower than 2.84 mmol/L. Thus, although hyper-responders increased their LDL-C during the high dietary cholesterol period, the resulting values are still within a range that is considered to be associated with a low risk. According to the updated clinical guidelines of the NCEP, a LDL/HDL ratio less than 2.5 is considered optimal [37]. In this study, values for LDL/HDL ratios, the best predictor for coronary heart disease risk, ranged from 1.62 to 1.80 for hyper-responders during the egg period, confirming that changes in lipoprotein profiles due to high levels of dietary cholesterol do not pose a risk for this population. However, the distinct individual responses to elevated dietary cholesterol and the subsequent changes in lipoprotein profiles need to be further examined in men and post-menopausal women, two groups who generally have a more adverse lipoprotein profile than the younger women examined in this study.

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