

Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population from northern Mexico¹⁻³

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ABSTRACT

Background: An increased incidence of coronary artery disease (CAD) is prevalent in northern Mexico. Effects of specific dietary components on risk factors for CAD have not been evaluated in children.

Objective: The purpose was to evaluate the effects of dietary cholesterol provided by whole eggs on the lipoprotein profile, LDL size, and phenotype in children from this region.

Design: Children (29 girls and 25 boys aged 8–12 y) were randomly assigned to either 2 eggs/d (EGG period; 518 additional mg cholesterol) or the equivalent amount of egg whites (SUB period; 0 additional mg cholesterol) for 30 d. After a 3-wk washout period, the children were assigned to the alternate treatment.

Results: Subjects were classified as hyporesponders (no increase or ≤ 0.05 mmol/L increase in plasma cholesterol for 100 mg additional cholesterol) or hyperresponders (≥ 0.06 mmol/L increase). During the EGG period, the hyperresponders ($n = 18$) had an elevation in both LDL cholesterol (from 1.54 ± 0.38 to 1.93 ± 0.36 mmol/L) and HDL cholesterol (from 1.23 ± 0.26 to 1.35 ± 0.29 mmol/L) with no changes in LDL:HDL. In contrast, hyporesponders ($n = 36$) had no significant alterations in plasma LDL or HDL cholesterol. All subjects had an increase in LDL peak diameter during the EGG period ($P < 0.01$) and a decrease ($P < 0.01$) in the smaller LDL subfractions. In addition, 5 of the children having LDL phenotype B (15%) shifted from this high-risk pattern to pattern A after the EGG treatment.

Conclusions: Intake of 2 eggs/d results in the maintenance of LDL:HDL and in the generation of a less atherogenic LDL in this population of Mexican children. *Am J Clin Nutr* 2004;80:855–61.

KEY WORDS Dietary cholesterol, children, LDL atherogenicity, LDL cholesterol, HDL cholesterol

INTRODUCTION

The multifactorial nature of coronary artery disease (CAD) comprises both nonmodifiable and modifiable risk factors. Dyslipidemias, including elevated concentrations of plasma LDL cholesterol and triacylglycerol, low HDL-cholesterol concentrations (1), and the presence of small dense LDL representative of the pattern B subclass (2), have a significant contribution to the incidence of CAD.

The primary treatment for modifying these risk factors should be changing dietary habits, increasing aerobic exercise, and maintaining body weight. Several of the dietary recommendations include decreasing saturated fat and cholesterol intake and

increasing complex carbohydrates and dietary fiber. However, a vast controversy surrounds dietary cholesterol and disease prevention (3). Furthermore, it is well known that remarkable variability exists in the response to dietary cholesterol among individuals; therefore, studies examining diet–gene interactions were carried out to explain the interindividual variability in the response to cholesterol intake (4).

A high prevalence of CAD, insulin resistance, and type 2 diabetes is found in northern Mexico (5). It is not clear whether the biomarkers for chronic disease can be identified at childhood or whether lifestyle choices over time play a significant role in the prevalence of chronic disease in the adult population. Therefore, studies aimed at understanding the cause of CAD and the contribution of modifiable risk factors to chronic disease are needed for this population.

An analysis of multiple cholesterol-feeding studies, conducted over a 50-y period, has shown that a modest increase in total cholesterol of 0.05–0.06 mmol/L could be predictable in response to a 100-mg/d increase in dietary cholesterol (6). If this moderate increase is used as a reference, those who experience elevations of ≥ 0.06 mmol/L in plasma cholesterol would be classified as hyperresponders, whereas hyporesponders would be those who have no change or have increases of ≤ 0.05 mmol/L in response to a 100-mg/d increase in cholesterol intake. In our previous reports in men and premenopausal women, the presence of 2 distinct populations was shown (7, 8). Therefore, it was important to identify in the pediatric population of the current study whether children can be classified similarly.

Although eggs are a central food in the diets of Mexicans that span all socioeconomic classes, little is known about the plasma lipid response to dietary cholesterol in the pediatric population. If egg intake does indeed have negative health implications, consumption would be expected to result in a detrimental lipoprotein profile and in the development of a more atherogenic LDL particle. Therefore, the purpose of the current investigation was to evaluate the effects of dietary cholesterol on plasma lipids

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and LDL atherogenicity in a pediatric population (children aged 8–12 y) from a region in Mexico where significant dyslipidemias were identified in the adults (9).

SUBJECTS AND METHODS

Materials

Enzymatic cholesterol and triacylglycerol kits were from Roche-Diagnostics (Indianapolis). Free cholesterol, apolipoprotein B (apo B), and apo E enzymatic kit was from Wako Pure Chemical (Osaka, Japan). EDTA, aprotinin, sodium azide, and phenyl methyl sulfonyl fluoride were obtained from Sigma Chemical (St Louis). Food scales were purchased at Ohaus Corporation (Pine Brook, NJ). Eggs were obtained from Pecuaría Sonorensis, Hermosillo, Sonora, Mexico, and marigold powder from Laboratories Castells, Mexico City.

Subjects and experimental design

The experimental protocol was approved by the University of Connecticut Institutional Review Board and by the Review Board of Centro de Investigación y Desarrollo A.C. Parents of children participating in the study attended informational meetings and signed the consent form. Children also received an explanation of the protocols in detail. Sixty children (30 boys and 30 girls) aged 8–12 y were recruited from the school Mauricio Kelly located in one of the lowest socioeconomic quarters of the city of Hermosillo, Mexico. They were randomly allocated to an egg (EGG) or a substitute (SUB)-based diet for a period of 30 d with a washout period of 3 wk followed by allocation to the alternate diet. Children consumed daily either the equivalent of 110 g or 2 whole eggs (518 additional mg dietary cholesterol) or the equivalent amount of egg whites (0 additional mg dietary cholesterol) with added color (SUB). Egg whites were colored with a commercial powder (10 mg/100 g egg), derived from marigold, which is rich in lutein.

Both the eggs and the SUB were prepared and served as scrambled eggs to all children every morning during breakfast in the school cafeteria. Children were divided into 2 groups, which had different sitting arrangements to avoid discussion on the potential differences in color between products (EGG compared with SUB). For the weekend, eggs were packed and sent home with the children. Parents were carefully instructed before the intervention started on feeding the correct product to the children, EGG or SUB (with the added color), during the corresponding periods. Parents were monitored closely and interviewed several times during the study to ensure that instructions were properly followed. Compliance was more than 98% both during the week and under parental supervision. In addition, to evaluate physical activity, children wore a pedometer attached to their clothing during a whole week to record the number of steps taken. Steps were recorded at baseline, during the EGG, and during the SUB periods. Fifty-four subjects (25 boys and 29 girls) completed the study.

Children were asked to follow their usual diet during both dietary periods. Three-day weighed food records (10) were used to evaluate dietary intake of macronutrients and dietary cholesterol during both periods. Both the children and the individual in charge of preparing meals at home were provided with food scales (Ohaus CS 2000) and a chart to record the subject's daily intake. Parents were instructed on how to keep the records, and

researchers in the study worked closely with children to document their food during the day. Diet intake was analyzed by using the ESHA Food Processor program (ESHA, Food Processor, 7.20, ESHA Research Editor, 1998, Salem, OR). Regional foods that were not included in the database were analyzed for individual components and added to the database. These foods include snacks, beans prepared in 3 different ways (dry, whole, and with broth), specific meats, flour and corn tortillas, organ meats, and candies, which were usually consumed by the children. Parents whose children agreed completed 3 24-h dietary records during each treatment period.

Plasma lipids and apolipoproteins

Two fasting (12 h) blood samples were collected on 2 different days for each subject into tubes containing 0.15 g/100 g EDTA to determine baseline plasma lipids. Plasma was separated by centrifugation at $1500 \times g$ for 20 min at 4 °C and placed into vials containing phenyl methyl sulfonyl fluoride (0.05 g/100 g), sodium azide (0.01 g/100 g), and aprotinin (0.01 g/100 g). Two additional blood samples were collected at the end of each diet treatment and washout period. The variables of weight, blood pressure, and level of activity were measured at baseline and after each dietary period to account for the possible influence of these factors on plasma lipids.

Total cholesterol was determined by enzymatic methods by using Roche-Diagnostics standards and kits (11). HDL cholesterol was measured in the supernatant after precipitation of apo B-containing lipoproteins (12) and LDL cholesterol was determined by using the Friedewald equation (13). Triacylglycerols were determined by using Roche-Diagnostics kits, which adjust for free glycerol (14). Means of the 2 blood draws were used to assess differences between treatment periods. Apo B concentrations were determined with use of an immunoturbidimetric method, and turbidity was measured in a microplate spectrophotometer at 340 nm (15). Apo C-III (16) and apo E (17) were measured with a Hitachi Autoanalyzer 740 using kits from Wako.

LDL size determination

The Lipoprint LDL system (Quantimetrix, Redondo Beach, CA), which uses nongradient high-resolution polyacrylamide gel electrophoresis, was used to determine LDL peak particle diameter and subclass distribution. Briefly, 25 μL plasma was added to precast polyacrylamide gel tubes and overlaid with 200 μL loading buffer. Tubes were then photopolymerized for ≈ 30 min 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 8.2–8.6) was added to the top and bottom portion of the chamber. The gel was run for ≈ 60 min at 36 mV or until the HDL fraction was ≈ 1 cm from the end of the tube. Gels were allowed to sit for 30 min and then scanned with a densitometer. The Lipoprint system quantifies 6 different LDL subclasses according to size. Most 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.



TABLE 1
Baseline characteristics of the boys and girls participating in the study¹

| Parameter | Boys (n = 25) | Girls (n = 29) |
|----------------------------------|---------------|-------------------------|
| Age (y) | 10.6 ± 1.6 | 10.2 ± 1.5 |
| Physical activity (steps/d) | 6624 ± 2893 | 6113 ± 2793 |
| BMI (kg/m ²) | 20.9 ± 4.3 | 18.4 ± 2.9 ² |
| Systolic blood pressure (mm Hg) | 102.5 ± 6.9 | 101.2 ± 5.3 |
| Diastolic blood pressure (mm Hg) | 64.8 ± 3.9 | 63.0 ± 3.2 |
| Total cholesterol (mmol/L) | 3.60 ± 0.44 | 3.46 ± 0.43 |
| LDL cholesterol (mmol/L) | 1.72 ± 0.48 | 1.69 ± 0.50 |
| HDL cholesterol (mmol/L) | 1.30 ± 0.22 | 1.22 ± 0.20 |
| Triacylglycerols (mmol/L) | 1.26 ± 0.37 | 1.21 ± 0.39 |
| LDL:HDL | 1.36 ± 0.43 | 1.45 ± 0.58 |

¹ All values are $\bar{x} \pm SD$.

² Significantly different from boys, $P < 0.05$ (Student's *t* test).

Data analysis

SPSS version 11.5 (SPSS Inc, Chicago) was used for statistical analysis. Student's *t* test was used to compare initial characteristics between boys and girls. Initial analysis revealed that there were no differences because of sex in the response to dietary cholesterol or to any of the measured parameters. Thus, to improve clarity in the presentation of data, boys and girls were pooled according to response classification for statistical analysis. Two-way analysis of variance ANOVA was used to analyze initial plasma lipids in boy and girl hyperresponders and hyporesponders. Repeated-measures ANOVA was used to analyze diet effects, responder effects, and the interactions for plasma lipids, apolipoproteins, dietary components, distribution of cholesterol in LDL subfractions, and LDL peak size. Each subject during both dietary periods was the repeated measure, whereas the comparison between groups was between hyperresponders and hyporesponders. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of the children [plasma lipids, level of activity, blood pressure, and body mass index (BMI)] classified by sex are presented in **Table 1**. No significant differences were seen in age between boys and girls. In addition, sex did not influence plasma total cholesterol, LDL cholesterol, HDL cholesterol, or triacylglycerol, level of activity, or blood pressure at baseline. However, BMI was higher in boys than girls.

Children in this population were characterized for having a value for HDL cholesterol in the 25th percentile according to the report from the National Cholesterol Education Program and triacylglycerol in the 95th percentile (18). In contrast LDL cholesterol and total cholesterol are in the 50th percentile for children in this age range (18).

As previously mentioned, a modest increase in total cholesterol of 0.05–0.06 mmol/L can be considered normal in response to a 100-mg/d increase in dietary cholesterol (19). On the basis of our data from our previous studies (7, 8) and our evaluation of the response in this group of Mexican children, subjects who experienced an increase in total cholesterol ≥ 0.06 mmol/L for each additional 100 mg dietary cholesterol were considered hyperresponders (**Figure 1**). Because children were fed an additional 518 mg/d dietary cholesterol during the EGG period, those children who experienced an increase in plasma cholesterol of ≥ 0.33

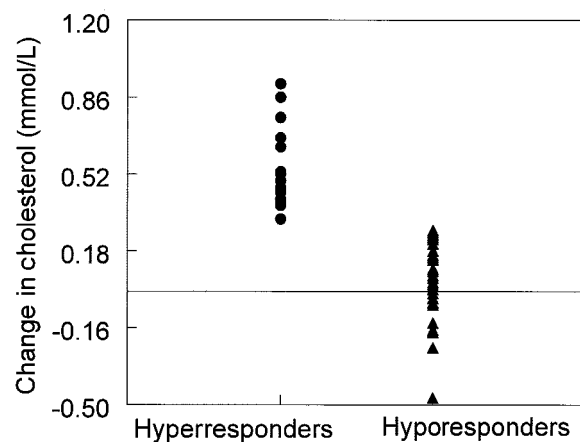


FIGURE 1. Changes in total cholesterol concentrations during the egg and the substitute periods for hyperresponders (●; n = 18) and hyporesponders (▲; n = 36).

mmol/L were considered hyperresponders. The remaining subjects who experienced fluctuations of <0.29 mmol/L (an increase in total cholesterol of ≤ 0.05 mmol/L for each additional 100 mg dietary cholesterol consumed) or had no change in plasma cholesterol were identified as hyporesponders.

After the classification of subjects, we analyzed the data to determine whether plasma lipids differed at baseline. Both boys and girls classified as hyporesponders or hyperresponders did not have significant differences in their baseline characteristics for plasma LDL, HDL, or triacylglycerol concentrations or for the other parameters evaluated, including level of exercise, blood pressure, and BMI (data not shown).

As shown in **Table 2**, this population of children was characterized by consuming a high-fat diet independent of dietary period. The percentage of energy derived from fat and saturated fat ranged from 37% to 43% and 10.5% to 13.6%, respectively. In contrast the percentage of carbohydrates was low, ranging from 42% to 49%. The high amount of fat in this population is mostly due to the consumption of low-cost meat products, including high-fat meat, chicken with skin, chorizo, and high-fat hot dogs plus hydrogenated oil used for the preparation of tortillas (20). However, the amount of dietary fiber in the diet is adequate and might be due to the high consumption of tortillas and beans (20).

In regard to response classification, the amount of total calories consumed was not different for hyperresponders or hyporesponders during both dietary periods. The percent of calories derived from carbohydrates and protein was higher during the SUB period ($P < 0.01$; Table 2). In addition, hyperresponders consumed less carbohydrate during the EGG period than hyporesponders during the EGG period or either group during the SUB period (interactive effect, $P < 0.05$). In contrast the percent of calories derived from fat and monounsaturated fatty acids (MUFAs) were higher during the EGG period ($P < 0.05$). Saturated fatty acid (SFA) was not significantly different in hyperresponders between diets. SFA was significantly lower during the SUB period in the hyporesponders than in the hyporesponders during EGG or than the hyperresponders during either period.

An interactive effect was also seen with hyperresponders consuming more total, SFA, and MUFA during the EGG period ($P <$

TABLE 2

Intakes of carbohydrate; protein; total fat; saturated (SFA), polyunsaturated (PUFA), and monounsaturated (MUFA) fatty acids; dietary cholesterol; dietary fiber; and vitamin E of hyper- and hyporesponders during the EGG and SUB periods¹

| Dietary component | Hyperresponders | | Hyporesponders | | P | | |
|----------------------------|-------------------------|---------------------------|-------------------------|-------------------------|-------------|------------------|-------------|
| | EGG | SUB | EGG | SUB | Diet effect | Responder effect | Interaction |
| Energy (kJ) | 6765 ± 1592 | 7182 ± 1610 | 7240 ± 1997 | 6583 ± 1797 | NS | NS | NS |
| Carbohydrate (% of energy) | 42.8 ± 3.8 ^b | 47.8 ± 8.3 ^a | 46.4 ± 7.6 ^a | 49.0 ± 7.5 ^a | < 0.01 | < 0.05 | < 0.05 |
| Protein (% of energy) | 14.4 ± 2.2 | 18.5 ± 3.1 | 15.0 ± 3.4 | 18.8 ± 3.9 | < 0.01 | NS | NS |
| Total fat (% of energy) | 43.1 ± 3.7 ^a | 38.3 ± 6.1 ^b | 39.2 ± 5.5 ^b | 37.1 ± 6.3 ^b | < 0.01 | < 0.05 | < 0.05 |
| SFA (% of energy) | 13.6 ± 2.0 ^a | 12.0 ± 3.0 ^{a,b} | 12.6 ± 2.4 ^a | 10.5 ± 2.4 ^b | < 0.01 | < 0.05 | < 0.05 |
| PUFA (% of energy) | 8.8 ± 1.9 | 7.6 ± 2.3 | 8.4 ± 2.3 | 9.2 ± 3.3 | NS | NS | NS |
| MUFA (% of energy) | 15.5 ± 2.2 ^a | 13.3 ± 3.2 ^b | 13.7 ± 3.0 ^b | 13.2 ± 2.8 ^b | < 0.05 | NS | < 0.05 |
| Dietary cholesterol (mg) | 602.8 ± 75.4 | 175.1 ± 144.3 | 663.8 ± 101.7 | 132.3 ± 67.6 | < 0.001 | NS | NS |
| Total fiber (g) | 19.3 ± 6.1 | 19.6 ± 7.9 | 16.6 ± 9.1 | 18.2 ± 7.5 | NS | NS | NS |
| Vitamin E (mg) | 14.6 ± 5.9 | 6.2 ± 3.1 | 14.8 ± 10.0 | 5.7 ± 2.2 | < 0.001 | NS | NS |

¹ All values are $\bar{x} \pm SD$ for $n = 18$ hyperresponders and 36 hyporesponders. During the EGG period, the subjects received 2 eggs (518 additional mg cholesterol)/d; during the SUB period, the subjects received egg whites (0 additional mg cholesterol). Within a row, values with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA and Tukey's post hoc test).

0.05) than any other group or time. The percent of calories derived from polyunsaturated fatty acids or the amount of total fiber were not different between dietary periods (Table 2).

As expected the amount of dietary cholesterol was significantly higher for all subjects during the EGG period and is mostly because of the 518 additional mg dietary cholesterol provided by the egg yolks (Table 2). Vitamin E was significantly higher during the EGG period, indicating the importance of whole egg in the contribution to the recommended values of 8 mg for girls and 10 mg for boys.

Other variables such as level of activity, fluctuations in body weight, or changes in blood pressure, which might influence plasma lipids, were analyzed during both dietary periods. As indicated in **Table 3**, no significant differences were seen in number of steps taken per day, systolic blood pressure, or BMI for hyporesponders or hyperresponders during the EGG or the SUB period. Interestingly, the diastolic blood pressure was lower during the EGG period for both hyperresponders and hyporesponders (diet effect, $P < 0.05$; Table 3).

Plasma lipids and apoproteins were evaluated after the classification of subjects as hyperresponders or hyporesponders. As shown in **Table 4**, there was a diet effect ($P < 0.001$) and

responder effect ($P < 0.001$) for both plasma LDL-cholesterol and HDL-cholesterol concentrations. There was also an interactive effect with the hyperresponders having higher concentrations of both LDL ($P < 0.01$) and HDL ($P < 0.05$) during the EGG period with no significant change in the hyporesponders. The total cholesterol:HDL (Table 4) and the LDL:HDL, important markers for coronary heart disease risk, were maintained during the EGG or SUB periods for all participants. The LDL:HDL was 1.49 ± 0.45 (EGG period) and 1.31 ± 0.41 (SUB period) for hyperresponders and 1.57 ± 0.49 (EGG period) and 1.56 ± 0.44 (SUB period) for the hyporesponders. In addition, plasma apo B concentrations did not differ between hyperresponders or hyporesponders or between EGG or SUB intake (Table 4). Likewise plasma apo C-III and apo E concentrations did not change during either dietary period. Values for apo C-III were 121 ± 31 (EGG period) and 115 ± 24 mg/L (SUB period) for hyperresponders and 106 ± 26 (EGG period) and 115 ± 37 mg/L (SUB period) for hyporesponders. For plasma apo E concentrations, values were 38 ± 14 (EGG period) and 33 ± 6 mg/L (SUB period) for hyperresponders and 35 ± 15 (EGG period) and 33 ± 8 mg/L (SUB period) for hyporesponders.

TABLE 3

Body mass index, systolic and diastolic blood pressure, and number of steps taken per day of hyper- and hyporesponders during the EGG and SUB periods¹

| | Body mass index | Systolic blood pressure | Diastolic blood pressure | No. of steps |
|------------------|-------------------|-------------------------|--------------------------|--------------|
| | kg/m ² | mm Hg | mm Hg | steps/d |
| Hyperresponders | | | | |
| EGG | 19.8 ± 4.1 | 100.2 ± 7.2 | 62.3 ± 3.2 | 6847 ± 3353 |
| SUB | 19.8 ± 3.9 | 100.3 ± 7.4 | 64.7 ± 4.0 | 6818 ± 2980 |
| Hyporesponders | | | | |
| EGG | 19.5 ± 3.6 | 102.9 ± 7.4 | 63.4 ± 4.5 | 6642 ± 3268 |
| SUB | 19.4 ± 3.8 | 101.9 ± 6.7 | 64.7 ± 5.2 | 6504 ± 3394 |
| Diet effect | NS | NS | $P < 0.05$ | NS |
| Responder effect | NS | NS | NS | NS |
| Interaction | NS | NS | NS | NS |

¹ All values are $\bar{x} \pm SD$ for $n = 18$ hyperresponders and 36 hyporesponders. During the EGG period, the subjects received 2 eggs (518 additional mg cholesterol)/d; during the SUB period, the subjects received egg whites (0 additional mg cholesterol). Repeated-measures ANOVA was used to analyze diet effects, responder effects, and their interaction.

TABLE 4

Plasma lipids, the ratio of total cholesterol (TC) to HDL, and apolipoprotein (apo) B concentrations of hyper- and hyporesponders during the EGG and SUB periods¹

| | LDL cholesterol | HDL cholesterol | TG | TC:HDL | Apo B |
|------------------|--------------------------|----------------------------|---------------|---------------|-------------|
| | <i>mmol/L</i> | <i>mmol/L</i> | <i>mmol/L</i> | <i>mmol/L</i> | <i>mg/L</i> |
| Hyperresponders | | | | | |
| EGG | 1.93 ± 0.36 ^a | 1.35 ± 0.29 ^a | 1.00 ± 0.68 | 2.85 ± 0.57 | 606 ± 96 |
| SUB | 1.54 ± 0.38 ^b | 1.23 ± 0.26 ^b | 1.02 ± 0.37 | 2.66 ± 0.52 | 598 ± 126 |
| Hyporesponders | | | | | |
| EGG | 1.88 ± 0.42 ^a | 1.28 ± 0.19 ^{a,b} | 0.93 ± 0.31 | 2.96 ± 0.55 | 583 ± 106 |
| SUB | 1.83 ± 0.44 ^a | 1.22 ± 0.19 ^b | 1.10 ± 0.47 | 3.00 ± 0.53 | 627 ± 109 |
| Diet effect | P < 0.0001 | P < 0.001 | NS | NS | NS |
| Responder effect | P < 0.001 | P < 0.001 | NS | NS | NS |
| Interaction | P < 0.01 | P < 0.05 | NS | NS | NS |

¹ All values are $\bar{x} \pm SD$ for $n = 18$ hyperresponders (9 boys and 9 girls) and 36 hyporesponders (16 boys and 20 girls). TG, triacylglycerol. During the EGG period, the subjects received 2 eggs (518 additional mg cholesterol)/d; during the SUB period, the subjects received egg whites (0 additional mg cholesterol). Within a column, values with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA and Tukey's post hoc test).

A detailed analysis of the LDL subfractions and LDL peak diameter is presented in **Table 5**. Of the 54 children who were analyzed 34 (63%) presented the pattern B associated with the small dense LDL (LDL-3) during the SUB period. However, 5 of the children shifted from pattern B to pattern A during the EGG period. LDL peak diameters were larger during the EGG period, and this finding was associated with increased concentration of LDL cholesterol in the largest fraction (LDL-1) ($P < 0.01$). The LDL-2 subfraction was not affected by diet or classification of response, whereas children had a greater concentration of the small LDL-3 subfraction during the SUB period. The lack of changes in apo B concentrations suggest that the number of LDL particles were not modified by diet (Table 4). These results agree with the observation of increased size in the LDL particles of hyperresponders during the EGG period.

DISCUSSION

The current recommendations for dietary cholesterol are based on epidemiologic data, which has shown a positive correlation between cholesterol intake and plasma cholesterol concentrations (21). However, epidemiologic studies need to account for additional confounding variables that are known to affect plasma lipids. For example, high dietary cholesterol can be

associated with an increase in consumption of SFAs and a decrease in the selection of fruit, vegetables, and foods high in fiber (3). Because children in the current study did not modify their level of activity during both dietary periods or had any fluctuations in body weight, it is logical to assume that diet had an important effect on the observed plasma lipids. Therefore, to study the response of this population of children to a dietary cholesterol challenge, we must first account for any possible effects of their high-fat intake on plasma lipid concentrations.

Diet analysis

The amount of fat consumed by the children was overall high and is representative of the regular intake in this region (20). The yolk-free substitute used in this study was void of fat and cholesterol; therefore, the variations observed in the amount and type of fat can be primarily attributed to the eggs consumed because 2 eggs provide 3.0 g SFA and 6.0 g MUFA (22). A strong positive relation exists between the percentage of energy obtained from SFAs and CAD incidence (21). Both hyperresponders and hyporesponders reported an increase in the percentage of energy from SFAs, suggesting that not only dietary cholesterol but also SFAs might contribute to the observed increases in LDL cholesterol in the hyperresponders. However, the hyporesponders had

TABLE 5

Distribution of cholesterol in LDL subfractions and LDL peak diameter of hyper- and hyporesponders during the EGG and SUB periods¹

| | LDL-1 | LDL-2 | LDL-3 | LDL peak diameter |
|------------------|---------------|---------------|---------------|-------------------|
| | <i>mmol/L</i> | <i>mmol/L</i> | <i>mmol/L</i> | <i>nm</i> |
| Hyperresponders | | | | |
| EGG | 1.52 ± 0.57 | 0.34 ± 0.19 | 0.16 ± 0.23 | 26.51 ± 0.10 |
| SUB | 1.16 ± 0.29 | 0.27 ± 0.13 | 0.26 ± 0.28 | 26.10 ± 0.11 |
| Hyporesponders | | | | |
| EGG | 1.45 ± 0.49 | 0.40 ± 0.22 | 0.24 ± 0.23 | 26.32 ± 0.09 |
| SUB | 1.37 ± 0.42 | 0.39 ± 0.14 | 0.25 ± 0.24 | 26.19 ± 0.09 |
| Diet effect | P < 0.01 | NS | P < 0.05 | P < 0.01 |
| Responder effect | NS | NS | NS | NS |
| Interaction | NS | NS | NS | NS |

¹ All values are $\bar{x} \pm SD$ for $n = 18$ hyperresponders and 36 hyporesponders. During the EGG period, the subjects received 2 eggs (518 additional mg cholesterol)/d; during the SUB period, the subjects received egg whites (0 additional mg cholesterol). Repeated-measures ANOVA was used to analyze diet effects, responder effects, and their interaction.

no significant modifications in their mean LDL-cholesterol concentrations during the EGG period. Part of these results could be explained on the basis of the type of fat consumed. Diets, which replace SFA with MUFA or polyunsaturated fatty acid, result in a decrease in plasma LDL-cholesterol concentrations (23) as much as 15% (24). In this study, hyperresponders were the only group that reported a significant ($P < 0.05$) increase in MUFA intake during the EGG period. These findings suggest that most of the fluctuations seen in the plasma compartment are driven by the egg's contribution of cholesterol to the diet. Other significant changes during the EGG period were a decreased intake of energy derived from carbohydrates and protein, which was mostly because of the replacement of these nutrients by dietary fat. Dietary fiber, a factor well known to influence plasma cholesterol concentrations (25), did not differ between periods.

Plasma lipids

In this study, boy and girl hyperresponders experienced significant increases both in LDL and HDL cholesterol as a result of the dietary cholesterol challenge, whereas plasma apolipoproteins were not altered. The observed increase in LDL cholesterol alone does not suggest that this population has increased their risk for CAD. Intravascular remodeling of lipoproteins as well as total cholesterol:HDL and LDL:HDL are also strong indicators of risk, and both ratios were maintained during both periods by the hyperresponders. In agreement with our results, other studies showed that elevations in plasma cholesterol attributed to dietary cholesterol are due to increases in the atherogenic LDL particle as well as the HDL fraction, which functions in a protective capacity (7, 8, 26–32). The findings of this study indicate that hyperresponders might increase their HDL cholesterol to promote the reverse cholesterol pathway by mobilizing the excess plasma cholesterol to the liver, the primary site of elimination from the body.


Dietary cholesterol and LDL atherogenicity

It is well established that the atherogenicity of the LDL particle is an important indicator of CAD risk. A predominance of LDL particles in the pattern B subclass was shown to be associated with a 3-fold increase in CAD risk (33, 34), which might be due to the facilitated uptake of this particle by the cells lining the arterial wall and also to its high susceptibility to oxidation (32). It is clear from the distribution of cholesterol toward the larger particles and the shift of some of the subjects from the phenotype B to the less atherogenic A, that the dietary cholesterol challenge reduced the atherogenicity of the LDL. These results become more important in light of the significant predominance of the phenotype B in this population of children. The lack of changes in apo B concentrations during the EGG period emphasized that the increase in LDL cholesterol in the hyperresponders was not due to an increase in the number of circulating particles, but to an increase in the size of the LDL.

Findings from twin studies contradict the possibility of complete genetic control over LDL phenotype (35) and suggest that genetic predetermination of LDL phenotype might be modifiable by environmental factors such as age, sex, adiposity, macronutrient composition of the diets, hormones, and drugs (36). In this study, we demonstrated that the macronutrient composition of eggs modified the phenotype by shifting some of the children to the pattern A. The frequency of the LDL pattern B phenotype in

the general population is approximately 30% in men and 15–20% in postmenopausal women (2). In the population from the current study, 64% of children were classified as having the B phenotype. Existing dietary prescriptions for the treatment and prevention of atherosclerosis and CAD are focused on reducing plasma LDL-cholesterol concentrations through the limitation of cholesterol and total fat intake with specific emphasis on restriction of SFA. Studies (37, 38) that examined the effects of such diets on lipoprotein concentrations showed a wide variation among individuals with some concluding that a low-fat and high-carbohydrate diet might actually increase risk by causing a general increase in plasma triacylglycerol (39) and decreased HDL cholesterol. This response was shown to be even 2-fold greater in persons with the pattern B phenotype (40). Thus, these concomitant fluctuations would negate any positive effects of the lowered LDL cholesterol achieved by the modification in macronutrient consumption. Because of the macronutrient composition of the diet during the egg period, a predominance of LDL-1 particles and a reduction of the smaller particles (+LDL-3) would be expected and was found in all children independent of response classification.

Plasma LDL-cholesterol concentration is widely used as a diagnostic tool for the prediction of atherosclerosis. However, plasma concentrations of individual lipoproteins might have restricted prognostic importance (33). Approximately 30% (41, 42) of patients with diagnosed premature CAD have plasma lipoprotein values that are within a range that is considered normal by National Cholesterol Education Program standards (43). In contrast, LDL subclass determination does appear to be a good predictor of atherosclerosis progression (44, 45). In fact, the Quebec Cardiovascular Cohort study (46), which examined 2034 men, found that a predominance of LDL particles with a peak diameter of <25.5 nm was positively associated with an increased risk of CAD (relative risk = 4.6, $P < 0.001$). If the traditional method for assessment of risk were to be used, the hyperresponders in this study would be expected to have an increase in risk after egg intake because of the resulting elevations in LDL cholesterol. However, this population was found to have a predominance of the larger LDL-1 subclass after egg consumption. These findings clearly illustrate the discrepancy that exists between the use of LDL-cholesterol concentrations and lipoprotein particle size as predictors of disease risk.

In conclusion, the results of this study clearly indicate that egg intake by a population of children with distinctive biomarkers for CAD and insulin resistance does not have negative health implications with regard to LDL:HDL or LDL atherogenicity. 

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