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Pilot Study on the Effect of Hyperimmune Egg Protein on Elevated Cholesterol Levels and Cardiovascular Risk Factors

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ABSTRACT

Coronary heart disease is the leading cause of death in the United States, accounting for almost half of all deaths. Animal studies have suggested that the daily addition of "hyperimmune egg" to one's diet might reduce the risk of cardiovascular disease. Military personnel with initial total cholesterol levels higher than 180 mg/dl were randomly enrolled in a 26-week double-blind study of a drink containing a hyperimmune egg protein. Subjects were randomly assigned to three groups: control (no drink); placebo (drink without egg); and active (drink combined with hyperimmune egg). Throughout the study this physically fit group maintained a program of strenuous exercise and participated in a dietary education program intended to reduce fat and cholesterol intake. At the end of the trial, total cholesterol levels of the control and placebo groups had increased, whereas the group that consumed the drink with hyperimmune egg showed no significant change in total cholesterol. The ratio of total cholesterol to high-density lipoproteins and the apolipoprotein B level increased in both control and placebo groups but remained essentially unchanged in the group consuming hyperimmune egg. Triglyceride and apolipoprotein A-I values did not change significantly in any of the groups. These findings suggest that hyperimmune egg may beneficially modify the regulation of serum lipoprotein levels and thereby reduce the possibility of cardiovascular disease.

INTRODUCTION

CORONARY HEART DISEASE (CHD) is the leading cause of death in the United States, accounting for almost half of all deaths (Department of Health and Human Services, 1986a, 1986b, American Heart Association, 1997). It is the second leading cause of death within the U.S. Military population (Department of the Army, 1987). Autopsy reports of American soldiers killed during the Korean and Vietnam wars revealed that 77% and 45%, respectively, of the casualties had evidence of atherosclerosis (Enos et al., 1953; McNamara et al., 1971).

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The National Cholesterol Education Program (NCEP) of the National Heart, Lung, and Blood Institute has recommended a population-based approach to detection and counseling of the general population as a preventive measure for CHD (National Institutes of Health, 1988). Large-scale epidemiological studies of the U.S. population have shown that CHD is strongly related to blood cholesterol levels (Dawber, 1980; Martin et al., 1986; Stamber, et al., 1986). It is documented that serum cholesterol levels increase with age in Western populations, resulting in widespread mild hypercholesterolemia from the age of 40 years onward (Berns et al., 1988). Senior enlisted soldiers display a similar trend, with increased cholesterol levels observed in more than 37% of students attending the U.S. Army Sergeants Major Academy at Fort Bliss, Texas, a training academy for career soldiers who have served at least 20 years in the Army. In addition, data collected during 5 years of health promotion efforts at the Academy indicate that the serum cholesterol levels of students rise moderately over the 9-month training period (Karge, 1996, unpublished observations).

Elevated blood cholesterol is a primary risk factor for CHD (Lipid Research Clinics Program, 1984; Castelli et al., 1986). Principal determinants of blood lipid and lipoprotein levels include genetic factors, dietary intake, and obesity. Major dietary factors affecting blood lipid and lipoprotein levels are total fat, saturated fat, cholesterol, and unsaturated fat (Mattson and Grundy, 1985). Intake of alcohol and fiber also affect lipid levels (Keys et al., 1965; Ulbricht and Southgate, 1991). Dietary cholesterol, which is incompletely absorbed, seems to have a lesser effect on blood cholesterol levels (Connor and Lin, 1974; Kris-Etherton et al., 1988). In addition, there appears to be wide variation in individual response to dietary cholesterol (McNamara et al., 1987).

Because of the risks of side effects associated with drug therapy, dietary intervention is usually the first treatment used in patients with mildly increased cholesterol levels (total cholesterol between 200 and 240 mg/dl) (National Institutes of Health, 1993). Long-term dietary changes that lower fat and cholesterol intake, as recommended by the American Heart Association (AHA) (1984), are difficult for many Americans to maintain (Kushner, 1993), as is the increased exercise prescription in most cholesterol reduction programs.

Consumers seeking alternatives to traditional protocols have increased their consumption of food products shown to reduce cholesterol, such as oat bran (Van Horn et al., 1988), fish oil (Daviglus et al., 1997), garlic (Warshafsky et al., 1993), and walnuts (Sabate et al., 1993). During 1994–1996, three fourths of Americans used reduced-fat or reduced-sugar snack foods (Department of Health and Human Services, 1996). In addition, antioxidant and vitamin supplementation has increased since these supplements were shown in some cases to reduce the risk of coronary artery disease (Stampfer et al., 1993; Hodis et al., 1995; Parthasarathy, 1998).

In unpublished studies, egg protein from chickens immunized with a polyvalent vaccine to multiple human gut bacteria decreased blood cholesterol levels by 16–33% and prevented coronary lesions in rabbits fed "hyperimmune" egg protein while consuming high-fat, high-cholesterol diets (W.H. Wilborn, 1992b, 1992c, 1992d, unpublished data). Humans who consumed milk from cows immunized with a similar polyvalent vaccine also had a significant reduction in total serum cholesterol (Golay et al., 1990; Sharpe et al., 1994). A significant blood pressure-lowering effect was also demonstrated in one of these studies (Sharpe et al., 1994). It therefore appears that hyperimmune egg protein has the potential to reduce cardiovascular risk and may prove to be an important alternative treatment for people at risk. The goal of this pilot study was to evaluate the potential for a commercially produced hyperimmune egg protein product to maintain healthy cholesterol levels and perhaps reduce cardiovascular risk.

MATERIALS AND METHODS

Subjects

This study was conducted using volunteers from the 475 students in the Sergeants Major Academy, Fort Bliss, Texas. Eighty-one volunteers were recruited from students who exhib-

ited elevated serum total cholesterol levels (180–290 mg/dl) during their entrance health screening. Forty-seven subjects, 44 men and 3 women with an age range of 35 to 55 years, completed all study activities.

Volunteers with hyperlipidemia secondary to thyroid disease, renal or hepatic dysfunction, obesity, or diabetes were excluded from the study. Volunteers were also excluded for medications and other conditions that would affect cholesterol levels or their measurement. Those subjects who did not complete all study activities voluntarily removed themselves from the study because the duration of the study became inconvenient or they lost interest in drinking one chocolate flavored beverage everyday.

Experimental design

The Human Use Review Committee of the U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts, approved the protocol. The length of the study was 26 weeks, and it was conducted on a free-living population. Volunteers continued their strenuous but typical physical fitness routine throughout the course of the study. Initial screening included lipid profiles, thyroid function panels, renal function panels, liver function panels, complete blood counts (CBCs), a blood pressure check, and medical and diet histories.

Volunteers in this double-blind study were randomly assigned to three treatment groups as follows (the numbers of subjects remaining at the conclusion of the trial are indicated): group I, untreated (control, no drink, dietary education, n = 15); group II, placebo (nutrient drink without egg, dietary education, n = 17); and group III, treated (nutrient drink combined with hyperimmune egg, dietary education, n = 15).

All students at the Academy were provided dietary education in health promotion classes conducted by the Academy Health Promotion Office and advised to follow a reduced-fat and -cholesterol diet similar to the AHA Step I diet (total fat intake approximately 30% of total calories; cholesterol intake approximately 300 mg/day). All groups consumed their normal diets obtained from a combination of meals at home, at restaurants, or at military dining facilities for 26 weeks. The soldiers in the drink groups also consumed their assigned beverage on a daily basis. Although there was no specific dietary intervention component to the trial, dietary intake was monitored during the 26 weeks of the study.

Dietary intake was evaluated by having volunteers record 3-day food diaries at the time of each blood draw. Not all volunteers completed dietary records owing to scheduling conflicts with their courses and other duties. Participants were asked to keep a record of all foods and beverages they consumed for 3 days at baseline, midpoint, and course completion. A trained dietary data collector reviewed records daily. Dietary intakes were analyzed with the use of software and the Moore's Extended Nutrient (MENu) database developed by the Pennington Biomedical Research Center, in combination with the 1988 SPSS statistical package (SPSS Inc., Chicago, IL). The nutrient database is a composite of the U.S. Department of Agriculture Nutrient Data Base Release for Standard Reference Release 10 (1993) and the Nutrient Data Base for Individual Food Intake Surveys (1994), both on CD-ROM, supplemented with manufacturers' data, data from the scientific literature, and other recognized food composition tables. Dietary intake was monitored to determine whether the diet changed significantly in any of the treatment groups and to verify consumption of the supplement in groups II and III.

The 26-week duration of the study was designed to coincide with the health promotion screening schedule during the soldiers' 9-month stay at the Academy. Blood samples were drawn for lipid profiles, liver function tests, CBCs, and cytokine assays at initial baseline, week 10, and week 26 of the study. Blood pressure was also measured at these intervals.

With the exception of group I (untreated), subjects were asked to return to the Academy Health Promotion Office every 2 weeks to obtain their supply of study product packets. To augment compliance, students were asked to turn in their empty and unused packets at each biweekly visit. All study subjects filled out health questionnaires at each biweekly visit to monitor for adverse experiences with the test product.

Study products

The placebo dietary supplement and the one containing spray-dried egg products from chickens immunized against a variety of gut bacteria were kindly provided by DCV BioNutrition (Wilmington, DE) and contained the following ingredients.

Dietary food supplement with hyperimmune egg. This product contained calcium caseinate, fructose, hyperimmune egg powder, corn syrup solids, partially hydrogenated soybean oil, emulsifiers (propylene glycol monoester, monoglycerides and diglycerides, sodium stearoyl lactylate) (for texture), microcrystalline cellulose, dipotassium phosphate, magnesium oxide, salt, natural and artificial flavors, adipic acid, aspartame, ascorbic acid, vitamin E, vitamin A palmitate, niacinamide, zinc oxide, electrolytic iron, copper gluconate, D-calcium pantothenate, vitamin D_2, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, vitamin B_{12}, folic acid, biotin, potassium iodide, and artificial color.

Dietary supplement without hyperimmune egg (placebo). This product contained calcium caseinate, fructose, corn syrup solids, partially hydrogenated soybean oil, emulsifiers (propylene glycol monoester, monoglycerides and diglycerides, sodium stearoyl lactylate) (for texture), microcrystalline cellulose, dipotassium phosphate, magnesium oxide, salt, natural and artificial flavors, adipic acid, aspartame, ascorbic acid, vitamin E, vitamin A palmitate, niacinamide, zinc oxide, electrolytic iron, copper gluconate, D-calcium pantothenate, vitamin D_2, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, vitamin B_{12}, folic acid, biotin, potassium iodide, and artificial color.

The nutritional composition of the study products was as follows: total calories, 70; calories from fat, 14; total fat, 1.5 g; saturated fat, 0 g; cholesterol, 35 mg; potassium, 100 mg; total carbohydrates, 3.5 g; sugars, 3.5 g; vitamin A, 10% of Daily Value; calcium, 15%; vitamin D, 10%; thiamine, 15%; niacin, 10%; folic acid, 10%; biotin, 10%; phosphorus, 10%; magnesium, 10%; copper, 10%; vitamin C, 15%; iron, 10%; vitamin E, 10%; riboflavin, 15%; pyridoxine, 15%; vitamin B_{12}, 10%; pantothenic acid, 10%; iodine, 10%; and zinc, 10%. The dietary supplement with the hyperimmune egg product was identical to the placebo with the exception that the addition of the 4.5 g egg represented an increase in cholesterol to a total of 85 mg.

Analytical techniques

All blood samples were collected after a 12-hour fast. Approximately 45 ml of blood was collected from each volunteer into tubes without anticoagulant for lipid profiles and liver function tests, and into tubes containing ethylenediamine tetraacetic acid (1 g/L) for CBCs. Serum was separated at room temperature and frozen for shipment to Pennington Biomedical Research Center, Baton Rouge, Louisiana. CBCs were performed on the day of the blood draw at William Beaumont Army Medical Center, Fort Bliss, Texas.

Cholesterol and triglyceride measurements and liver and thyroid function tests were performed by commercial enzymatic methods. High-density lipoprotein (HDL) cholesterol was measured after apolipoprotein (Apo) B-containing lipoproteins had been precipitated from serum with dextran sulfate. The combination of very-low-density, intermediate-den-

TABLE 1. DESCRIPTIVE CHARACTERISTICS FOR EACH DRINK GROUP

Characteristic	*Egg drink*	*Placebo*	*Untreated*
Number	15	17	15
Age	40.8 ± 4.8	42.9 ± 5.4	41.5 ± 3.7
Height (cm)	177.0 ± 6.9	171.5 ± 8.6	176.0 ± 6.4
Weight (kg)	81.4 ± 11.4	80.0 ± 11.0	82.2 ± 9.2
Body mass index	26.0 ± 2.7	27.3 ± 2.7	26.7 ± 2.8
Total cholesterol (mg/dl)	224 ± 22	222 ± 26	234 ± 36

Values are mean ± SD.

TABLE 2. PHYSICAL FITNESS INDICES (AFPT SCORES) FOR EACH DRINK GROUP

Item	*Baseline*	*Final*
Number of sit-ups		
Egg drink	64 ± 13	65 ± 15
Placebo	60 ± 18	58 ± 17
Untreated	60 ± 16	57 ± 13
Number of push-ups		
Egg drink	52 ± 16	55 ± 18
Placebo	50 ± 18	52 ± 14
Untreated	51 ± 19	54 ± 14
Run time (min)		
Egg drink	14.6 ± 1.7	14.9 ± 1.8
Placebo	14.9 ± 2.1	14.9 ± 1.3
Untreated	14.8 ± 1.2	15.3 ± 1.4

Values are mean ± SD. No. subjects by group: egg drink, 12; placebo, 13; untreated, 10.

sity, and low-density lipoprotein (LDL) cholesterol levels was calculated by subtracting HDL cholesterol from total cholesterol. Because of the minimal contribution of very-low-density and intermediate-density lipoproteins to these values, the measurement of non-HDL cholesterol is referred to as LDL cholesterol. Apo A-I and B were determined by rate nephelometry. Measurements of the cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) and of soluble selectins (E-, L-, and P-selectin) were made by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Statistical analysis

Intervention study data were analyzed using a one-way repeated-measures analysis of variance (ANOVA), with a probability level of $P < .05$ considered significant. All data are reported as mean ± standard deviation (SD). Measurements that showed a significant F value over time were further analyzed for mean differences from baseline with the use of Tukey's Honestly Significant Difference (HSD) multiple comparison test. Measurements made only at

TABLE 3. LIPID METABOLISM INDICES FOR EACH DRINK GROUP

Item	*Baseline*	*Midpoint*	*Final*
Total cholesterol (mg/dl)			
Egg drink	224 ± 22	228 ± 23	237 ± 38
Placebo	222 ± 26	233 ± 34	247 ± 49[1]
Untreated	234 ± 36	248 ± 54	249 ± 53[2]
Triglycerides (mg/dl)			
Egg drink	101 ± 53	94 ± 56	101 ± 62
Placebo	101 ± 47	99 ± 40	109 ± 47
Untreated	121 ± 92	116 ± 48	140 ± 62
HDL cholesterol (mg/dl)			
Egg drink	51.0 ± 13.1	53.5 ± 14.9	52.2 ± 12.6
Placebo	48.5 ± 8.9	49.4 ± 10.3	50.9 ± 10.1
Untreated	44.9 ± 9.5	45.1 ± 8.0	42.3 ± 10.5[3]
LDL cholesterol (mg/dl)			
Egg drink	151.7 ± 18.9	153.9 ± 25.2	161.0 ± 34.1
Placebo	152.9 ± 20.1	163.6 ± 29.1	173.8 ± 45.3
Untreated	163.5 ± 38.2	180.6 ± 57.4	175.9 ± 55.8
TC:HDL ratio			
Egg drink	4.7 ± 1.3	4.6 ± 1.4	4.8 ± 1.4
Placebo	4.7 ± 0.9	4.9 ± 1.1	5.0 ± 1.3[2]
Untreated	5.5 ± 1.6	5.7 ± 1.5	6.1 ± 1.5[1]
Apo A1 (mg/dl)			
Egg drink	152.5 ± 23.8	136.2 ± 20.4	151.0 ± 25.0
Placebo	148.4 ± 18.4	130.0 ± 14.8	155.2 ± 19.3
Untreated	143.3 ± 19.2	127.7 ± 13.1	146.3 ± 17.6
Apo B (mg/dl)			
Egg drink	119.4 ± 18.4	124.2 ± 25.4	125.5 ± 24.8
Placebo	121.2 ± 17.1	131.0 ± 25.5	143.1 ± 38.2[1]
Untreated	127.9 ± 32.0	136.3 ± 35.8	154.2 ± 41.1[1]

Values are mean ± SD. No. subjects by group: egg drink, 15; placebo, 17; untreated, 15.
[1]Significantly different from baseline ($P < .05$).
[2]Different from baseline ($P < .10$).
[3]Significantly different from other treatment groups ($P < .05$).

the beginning and end of the study were evaluated by Student's paired-sample t test. Statistical analyses were performed with the use of the SPSS statistical software package (SPSS).

RESULTS

Health screen

Descriptive characteristics and initial serum cholesterol levels of volunteers are presented in Table 1. Initial serum total cholesterol (TC) ranged from 184 to 292 mg/dl for the entire test group (n = 47). No significant differences were found among the three treatment groups for liver, thyroid, or renal function. Similarly, CBC parameters and systolic and diastolic blood pressure values showed no significant changes. All volunteers met the U.S. Army standards for body fat and weight at baseline and throughout the entire 26-week trial period. Men maintained a body mass index (BMI) of 26 or less, and women maintained a BMI of 36 or less, in accordance with Army regulations. All volunteers participated in scheduled physical exercise and were at a very high level of fitness as judged by their scores on the Army Physical Fitness Test (APFT) (Department of the Army, 1998). These career soldiers had been following a standard military exercise program for a minimum of 15 years and did not start an exercise program significantly different from what they had practiced before arriving at the Academy. This exercise program usually included a minimum of 3 days of aerobic exercise weekly, typically running at least 2 miles per exercise period.

Compliance and adverse experiences

In this trial, the dietary supplement by itself and the supplement combined with egg were well tolerated, with 17.9% of the hyperimmune egg treatment group, 13.8% of the placebo group, and 5% of the untreated group reporting diarrhea, gas, and bloating. The mild gastrointestinal symptoms were transitory in nature, and none required medical intervention. Compliance over the study period was high, with a mean compliance of 82% in both the placebo group and the hyperimmune egg treatment group. The consumption of the nutrient drinks did not adversely affect the rigorous training or exercise regimens of the volunteers, as evidenced by the comparable APFT scores in the three treatment groups (Table 2). Not all of the 47 subjects completed every event in the APFT, so the sample sizes are different in each group. Changes in body weight over time in the egg drink group (0.10 ± 1.35 kg) and in the placebo group (−0.64 ± 1.86 kg) were not statistically significant.

Lipid levels

Lipid variables for the three groups are summarized in Table 3. There were no significant differences in TC, triglycerides, LDL, or Apo A among treatment groups or over time, as analyzed by repeated-measures ANOVA. When the data were analyzed by the paired t test, however, TC was found to increase significantly from baseline to endpoint in the placebo group ($P < .004$) and demonstrated a trend toward increasing in the untreated group ($P < .10$). In contrast, TC did not increase significantly over time in the hyperimmune egg treatment group. The only significant difference in HDL was a lower endpoint HDL in the untreated group compared with the placebo group ($P < .05$).

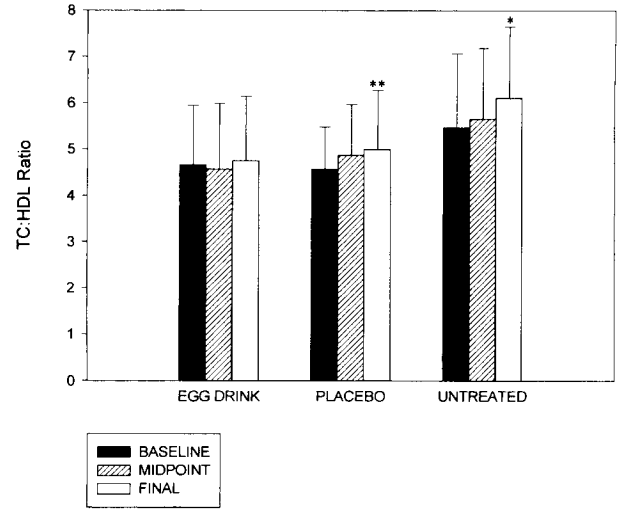

FIG. 1. TC:HDL ratio for each treatment group over time: egg drink (n = 14), placebo (n = 16), untreated (n = 14). Values are mean ± SD. *Significantly different from baseline ($P < .05$). **Different from baseline ($P < .10$).

Analysis by repeated-measures ANOVA showed a significant time ($P < .02$) and drink ($P < .05$) effect for the ratio between TC and (TC:HDL ratio). A *post hoc* analysis using Tukey's HSD test indicated that the significant findings could be attributed to an increase in the ratio in the untreated group. Additional variability was accounted for by a significant difference ($P < .04$) between the TC:HDL ratio in the hyperimmune egg treatment group and in the untreated group at the endpoint evaluation (Fig. 1). The TC:HDL ratio tended to increase over time in all groups, but this increase was less in the hyperimmune egg treatment group (Fig. 2).

Another finding was a stabilization of Apo B secretion over time in the egg treatment group. Apo B showed a significant time effect ($P < .001$) when analyzed by repeated-measures ANOVA. When analyzed by paired t test, Apo B was found to increase significantly from baseline to endpoint in the placebo group ($P < .02$) and in the untreated group ($P < .004$), whereas the increase over time in the egg treatment group was not significant (Fig. 3). This phenomenon is further illustrated by examining the change in Apo B over time, as shown in Figure 4.

Immune function

Only a small sample size was available for laboratory and statistical analyses (n = 7 for the untreated group, n = 10 for the placebo group, and n = 10 for the egg treatment group) due to serum collection problems. Although IL-6 cytokine levels tended to be lower in the egg treatment group at the midpoint and final evaluations (Fig. 5), no significant differences were found in white blood cell count; cytokines IL-1, IL-6, and TNF-α; and the soluble selectins E-, L-, and P-selectin, measured as indices of

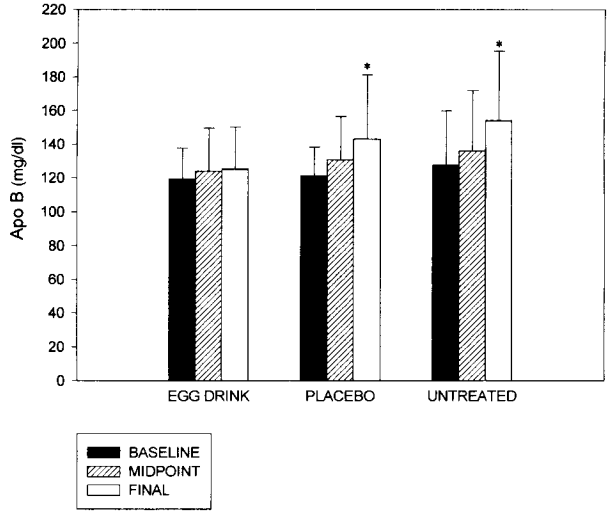

FIG. 3. Apo B levels for treatment groups over time: egg drink (n = 10), placebo (n = 14), untreated (n = 11). Values are mean ± SD. *Significantly different from baseline ($P < .05$).

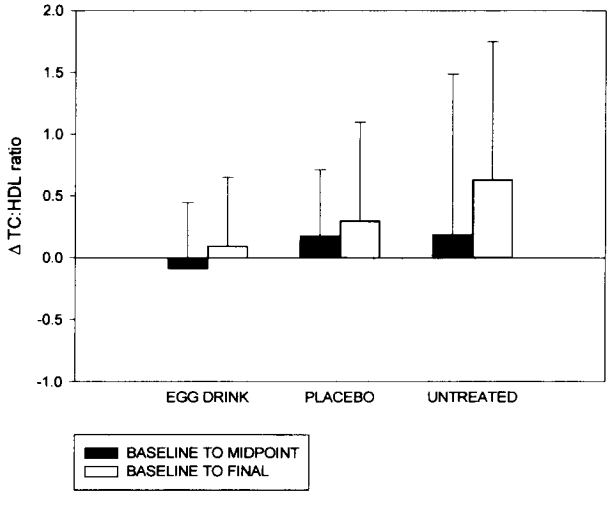

FIG. 2. Changes in TC:HDL ratio for each treatment group over time: egg drink (n = 14), placebo (n = 16), untreated (n = 14). Values are mean ± SD.

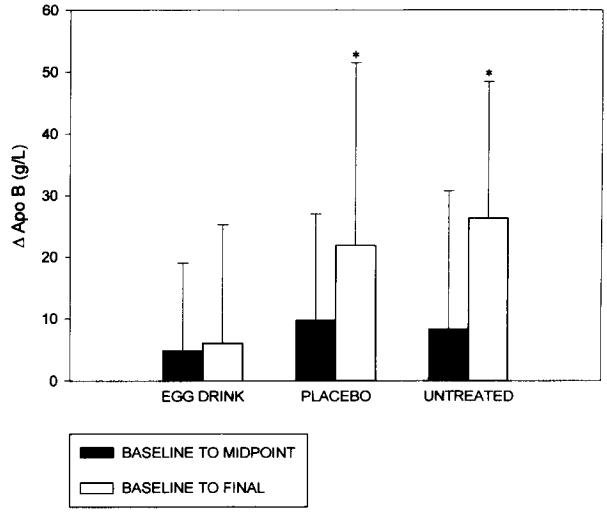

FIG. 4. Changes in Apo B levels for each treatment group over time: egg drink (n = 10), placebo (n = 14), untreated (n = 11). Values are mean ± SD. *Significantly different from baseline ($P < .05$).

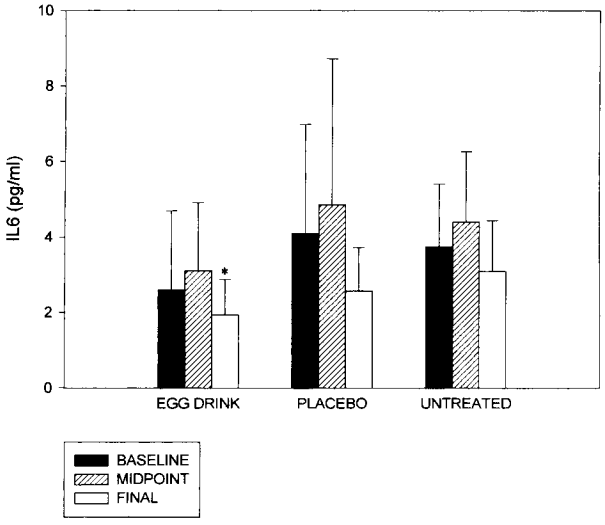

FIG. 5. IL-6 levels for each treatment group over time: egg drink (n = 10), placebo (n = 10), untreated (n = 7). Values are mean ± SD. *Different from untreated final ($P < .10$).

immunological status. The small sample sizes for these measurements may have contributed to the inability to detect any statistically significant changes.

Diet

Dietary intake records were completed by a subset of volunteers for the study; therefore, the sample sizes were smaller for each group (Table 4). Because of the large variability in energy intake among subjects, there were no significant differences in total caloric intake among the three groups. However, for reasons not understood, the egg treatment group did not decrease their total caloric intake as much as the other two groups did. In addition, the egg treatment group consumed significantly more calories from saturated fat ($P < .05$) than the untreated group at the midpoint evaluation. However, this difference disappeared by the time of the final dietary intake evaluation. The egg treatment group consumed significantly more cholesterol per day than did the untreated group ($P < .05$) after 10 weeks of the study and more than the placebo group after 26 weeks of the study ($P < .05$). These differences in intake are greater than the additional 50 mg of cholesterol consumed daily in the egg treatment beverage.

DISCUSSION

The data obtained in this pilot study show that consumption of a hyperimmune egg product by a physically fit group of subjects blunted an expected rise in total serum cholesterol levels and lowered cardiovascular risk ratios (TC:HDL). Increases in TC levels in the

TABLE 4. DIETARY INTAKE VALUES FOR EACH DRINK GROUP

Item	*Baseline*	*Midpoint*	*Final*
Total energy (kcal/d)			
Egg drink	2570 ± 714	2781 ± 1035	2574 ± 722
Placebo	2577 ± 478	2239 ± 628	1832 ± 901
Untreated	2673 ± 482	2332 ± 793	2026 ± 522
Cholesterol (mg/d)			
Egg drink	394 ± 208	376 ± 176[1,4]	295 ± 151[3]
Placebo	306 ± 154	232 ± 122	119 ± 93
Untreated	349 ± 112	140 ± 105	245 ± 58
Total fat (% energy)			
Egg drink	34.69 ± 9.57	33.90 ± 5.47[2]	29.45 ± 5.17
Placebo	34.38 ± 5.18	31.40 ± 4.66	28.41 ± 4.39
Untreated	34.31 ± 4.85	26.14 ± 10.27	29.96 ± 5.97
Saturated fat (% energy)			
Egg drink	11.99 ± 4.31	11.11 ± 1.87[4]	9.57 ± 2.70
Placebo	11.79 ± 3.32	10.51 ± 1.59	8.84 ± 1.97
Untreated	11.52 ± 2.84	8.08 ± 3.06	10.86 ± 2.30

Values are mean ± SD. No. subjects by group: egg drink, 9; placebo, 12; untreated, 6.
[1]Different from placebo ($P < .10$).
[2]Different from untreated ($P < .10$).
[3]Significantly different from placebo ($P < .05$).
[4]Significantly different from untreated ($P < .05$).

placebo group ($P < .004$) and in the untreated (control) group ($P < .10$) were observed over the 26-week period. In contrast, those subjects consuming the dietary supplement with hyperimmune egg did not experience a statistically significant increase in TC. The 7–10% increase in TC observed in the placebo and untreated groups is consistent with unpublished observations of students from 5 years of health promotion efforts at the Academy. In addition, this student class exhibited a trend similar to that seen in previous classes, with the TC increasing by an average of 10% in the total student population (n = 475).

The hyperimmune egg portion of the drink consumed in this pilot trial was a total of 4.5 g/day (equivalent to one third of a whole egg), compared with the 70 g/day of milk used in the studies of Sharpe et al. (1994) and Golay et al. (1990). In a type II collagen model of arthritis in rats, the oral administration of hyperimmune egg powder significantly decreased the incidence and severity of arthritis in a dose-dependent manner (Trentham, et al., 1998). Therefore, a greater serving of hyperimmune egg might have provided an even greater cardiovascular benefit than was seen in this trial and warrants further study.

Several epidemiological studies have indicated that the TC:HDL ratio is a strong predictor of CHD, with the recommendation that the ratio be modified to 4.5 or lower to reduce risk for CHD (Castelli, 1984; Grundy et al., 1987). In this pilot study, TC:HDL ratio was one of the two variables (the other being Apo B) that showed the greatest difference between the treatment group and the control and placebo groups. Whereas the untreated and placebo groups showed a gradual increase in TC:HDL ratio over time, the group consuming the hyperimmune egg maintained a fairly stable TC:HDL ratio. These results suggest that consumption of hyperimmune egg may reduce cardiovascular risk.

Although not routinely applied in clinical practice, clinical trial data suggest that modification of Apo B levels may reduce the risk for CHD (Fager et al., 1981; Riesen et al., 1980). A major constituent of the Apo B particle is LDL cholesterol, which is used clinically as an independent risk factor for CHD. In this study, Apo B showed increases over time in all groups but was only slightly increased in the egg treatment group. Whereas Apo B increased gradually through the midpoint to the final screen in the placebo and untreated groups, this marker remained stable in the egg treatment group.

The final TC:HDL ratio and the final HDL concentration of the untreated and placebo groups remain unexplained. Perhaps the base placebo nutrient drink had an effect or the placebo group was altering their diet and behavior in some way. The placebo group may have been more involved in monitoring their diet and behavior as a result of consuming the beverage packets every day. An alternative explanation is that they altered their diet as a result of consuming the drink by eating fewer high-fat or high-cholesterol foods to compensate for the added calories and bulk of the nutrient drink. Although there were no significant differences in dietary intake in the three groups, several trends were noted. The untreated group tended to increase cholesterol consumption at 26 weeks to a level greater than that in the placebo group and closer to the higher level in the hyperimmune egg treatment group. In addition, the portion of kilocalories consumed from saturated fat tended to decrease as time went on in the placebo group, whereas in the untreated group it remained close to the baseline intake level.

The mechanisms for the changes in lipid concentrations and decreases in cardiovascular risk observed in this trial are intriguing. One potential mechanism is a systemic effect of some component of the hyperimmune egg on cholesterol metabolism. The significant changes in Apo B levels in the untreated and placebo groups indicate that the production or catabolism of this protein may be altered by consumption of the hyperimmune egg. Cholesterol metabolism in smooth muscle cells and macrophages and cholesterol trafficking have been shown to be affected by immunomodulatory factors released during vascular inflammation (Hajjar and Pommerantz, 1992). Also, TNF, granulocyte-macrophage colony-stimulating factor, and IL-2 modulators lower serum cholesterol in humans (Sherman et al., 1988; Wilson et al., 1989). Interferon, another immunoregulatory factor, has been shown to in-

hibit atherogenesis in rabbits (Wilson, et al., 1990). Additionally, it has been proposed that these cytokines may promote cholesterol deposition and clearance by the spleen and other reticuloendothelial cell beds, thereby decreasing the amount of plasma LDL cholesterol and reducing the net cholesterol load to the vasculature.

One of the biological responses observed in atherosclerosis is the accumulation of lymphocytes and macrophages at sites of plaque formation in blood vessels. These cellular interactions are fundamentally no different from those seen in other inflammatory diseases (Ross, 1999). The mechanism of recruitment of such cells has not been delineated; however, involvement of immunoregulatory products is suggested (Nelken et al., 1991). A low-molecular-weight fraction isolated from the milk of hyperimmunized cows (HIMF) appeared to inhibit localized inflammation by suppressing carrageenan-induced neutrophil migration (Ormrod and Miller, 1991, 1992, 1993). Hyperimmune egg has been shown to act in a similar fashion (W.H. Wilborn, 1992a, unpublished data). The ability of neutrophils to respond to chemotactic stimuli and to adhere to endothelial cells was not affected in these studies. Ormrod and Miller hypothesized that HIMF could downregulate the synthesis of proinflammatory cytokines or adhesion molecules, because they produced similar results to those obtained when cells were treated with cyclosporine, which influences cytokine production.

It has been suggested that induction of atherosclerosis may be mediated by injury or by pathogenic organisms such as *Chlamydia* (Jackson et al., 1997). Because hyperimmune egg may decrease the migration of mononuclear cells into an area where an atherosclerotic plaque is forming, it is possible the egg protein could in this manner assist in reducing atherosclerotic plaque formation.

This study attempted to look at such a mechanism by measuring the levels of the cytokines IL-2, IL-6, and TNF-α, along with the levels of selectins that serve a role in neutrophil adhesion (P-, E-, and L-selectin). Perhaps because of the transient nature of such factors in the circulation, no significant changes were demonstrated. C-reactive protein is a marker for systemic inflammation and has been shown to predict the risk of future myocardial infarction (Ridker et al., 1997). Most of the subjects in the present study had a C-reactive protein level below the lower limit of detection for the assay. In this study, IL-6 was the only immunological parameter that showed any change. Subjects consuming the hyperimmune egg product showed a trend toward a slight decrease in IL-6 level.

Taken in its totality, the evidence acquired in this pilot study suggests that immune egg may contain immunoregulatory components that inhibit certain cardiovascular events.

Another mechanism that may explain the blunting of cholesterol rises seen in the hyperimmune egg treatment group involves a local effect of antibodies in the immune egg on gastrointestinal flora. Alterations in the flora could result in increased bile acid and cholesterol excretion or in decreased cholesterol absorption. Specific antibodies from egg or milk have been shown to provide protection against pathogens with which the host has been vaccinated (Davidson et al., 1989; Boesman-Finkelstein and Finkelstein, 1991; Hammarström et al., 1994). The evidence is strong that antibodies survive digestion in the stomach and maintain their localized activity in the intestine (Hammarström et al., 1994). If these antibodies decrease the colonization of the intestine by pathogenic organisms, then it is likely that they alter floral ratios in the intestine, which might affect cholesterol levels.

Results obtained in this study must be viewed in the context of the specific military population studied. Throughout the 26 weeks of study this physically fit, nonobese group maintained an active program of strenuous exercise. Dietary interviews indicated that the students had reduced their fat and cholesterol intakes to levels recommended by the AHA. Therefore, the majority of this physically fit population were already maintaining a regimen associated with reductions in serum cholesterol levels without pharmacologic intervention.

The rise in cholesterol levels in the class as a whole, and in the untreated and placebo groups, despite the exercise program and healthy diet, are difficult to understand and yet

have been observed for the last 5 years (W.H. Karge, unpublished observations). The consistency of this history of cardiovascular risk was one of the primary reasons that this population was chosen for this pilot study. The increases in CHD risk in this population suggest that there are substantive differences in the lifestyles of these subjects while at the Academy, compared with their previous assignment. Decreased exercise levels over time as the class load increased could account for some of the increased values. Daily physical activity over the weeks of classes was not monitored. Several students who experienced increases in their serum cholesterol levels provided anecdotal reports stating they did not exercise as much as they had at their previous military post.

Any positive change in lipid levels resulting from egg protein consumption could have been more difficult to observe given that this population may already have achieved the maximum possible reductions to be obtained by nonpharmacologic interventions. Decreasing dietary fat and cholesterol intake, along with starting a new exercise program or increasing the amount of exercise performed, is a standard practice in the treatment of patients with mild to moderate hypercholesterolemia. The fact that even a modest change was measured clearly supports the need for further research in a more mainstream population of less active hypercholesteremic patients, where the potentially cardioprotective effects of the immune egg protein can be more readily observed.

In conclusion, the preliminary findings of this trial support the contention that consumption of hyperimmune egg product inhibits a rise in serum cholesterol and thereby decreases risk for cardiovascular disease. In contrast to the hyperimmune egg group, the TC levels in the untreated and placebo groups increased significantly over time. In addition, other potential risk factors for cardiovascular disease, such as the TC:HDL ratio and the Apo B level, increased over time in the two groups that did not receive the immune egg product. Egg products from hyperimmunized chickens, when incorporated into a lipid-lowering dietary regimen, may prove to be an effective supplement for persons with moderately increased cholesterol levels.

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