

Cholesterol-lowering effects of bovine serum immunoglobulin in participants with mild hypercholesterolemia¹⁻³

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ABSTRACT

Background: The consumption of milk products has been shown to lower cholesterol. The mechanism of action surrounding this observation has been attributed to the protein fraction of milk and serum.

Objective: We examined the effect of an oral serum bovine immunoglobulin protein fraction (bIg) derived from US Department of Agriculture–approved beef (aged <30 mo) on lipid indexes in humans.

Design: Participants included men and women (aged 25–70 y) with hypercholesterolemia (5.44–6.99 mmol/L) who were not receiving cholesterol-lowering medication. Treatment consisted of the randomized, double-blind, parallel-group, placebo-controlled administration of 5 g bIg for 6 wk in 52 participants ($n = 26$ each in treatment and control groups).

Results: Mean (\pm SD) total cholesterol (TC) at baseline was 6.33 ± 0.1 mmol/L for bIg and 6.16 ± 0.1 mmol/L for placebo. A repeated-measures multivariate analysis of covariance covaried for change in total energy and alcohol intake and Tukey's post hoc examination of our data showed that the bIg-treated group had a significant reduction in TC at 3 wk (5.98 ± 0.5 mmol/L; $P < 0.05$) and 6 wk (5.97 ± 0.7 mmol/L; $P < 0.05$). The concentration at 6 wk was significantly different from that in the placebo group ($P < 0.05$). This reduction was largely due to a decrease in LDL cholesterol in the bIg-treated group from baseline (4.12 ± 0.6 mmol/L) at 3 wk (3.92 ± 0.7 mmol/L, $P < 0.05$) and at 6 wk (3.84 ± 0.6 mmol/L, $P < 0.05$); the 6-wk concentration differed significantly between the treatment and placebo groups ($P < 0.05$). We observed no significant changes in the placebo group or in any other lipid indexes or markers associated with hepatorenal or cardiovascular function.

Conclusion: Consumption of bIg appears to positively modulate the primary lipid indexes associated with cardiovascular disease. *Am J Clin Nutr* 2005;81:792–8.

KEY WORDS Cholesterol, cardiovascular disease, LDL, lipids, bovine serum immunoglobulin, bIg

INTRODUCTION

Hypercholesterolemia is an important risk factor for cardiovascular disease. Accordingly, guidelines from the National Cholesterol Education Program suggest weight reduction and dietary management as preferred alternatives for lowering cholesterol and managing heart disease (1). Barring lifestyle changes, the use of medications such as statin drugs is effective,

although some persons experience negative sequelae that contraindicate their use (2, 3). Thus, a continual investigation for alternative solutions is warranted. An area for potential investigation is the constituents found in milk (4–7), although the mechanism of action surrounding milk consumption has not been adequately identified (8). Of the principal proteins present in milk, those found in whey protein present an interesting avenue for investigation, because casein is actually thought to be hypercholesterolemic when compared with other protein sources (9, 10). Moreover, 2 studies have reported that the administration of 90 g dried skim milk from hyperimmunized cows lowers cholesterol in humans with mildly elevated cholesterol concentrations (11, 12). In those studies, the primary difference between the control skim milk and the skim milk from hyperimmunized cows was the protein content of bovine immunoglobulin (bIg).

Whereas milk and whey contain low concentrations of bIg (<1% and 3–4%, respectively), immunoglobulin comprises at least 25% of the protein content of serum. This is an important consideration because the intake of 90 g skim milk solids may be impractical for consumers. However, the purification of bIg from US Department of Agriculture (USDA)–approved edible bovine serum produces a higher bIg concentration so that persons can consume smaller quantities. Serum protein also contains other factors that could alter cholesterol absorption. These factors include antibodies that are reactive with cholesterol and lipoproteins (13), which might interfere with cholesterol absorption (14). In an 8-wk pilot study (E Weaver, C Siefken, and R Strohhahn, unpublished observations, 2002), our group found that participants with mild hypercholesterolemia showed a significant reduction in total cholesterol (TC) after the administration of 5 g bIg/d. The objective of our current trial was to examine bIg supplementation on blood lipids in a larger cohort by using a formal randomized, double-blind clinical trial.

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SUBJECTS AND METHODS

Study population

We examined 52 men and women aged 25–70 y who volunteered for this investigation, which was conducted at The Cooper Institute Center for Human Performance and Nutrition Research (Dallas, TX). All participants had hypercholesterolemia (5.44–6.99 mmol/L). We recruited persons from the surrounding Dallas and Ft Worth communities by radio, television, and newspaper advertisements. All participants entering the trial provided written informed consent as approved by The Cooper Institute Institutional Review Board before entering into the investigative portion of our trial. Participants were excluded from the trial if their body mass index (BMI; in kg/m²) was <18.5 or >30, if they had recently donated blood (<3 mo), or if they did not agree not to donate blood during the trial. Also excluded were participants with elevated blood pressure, TC, LDL cholesterol, or fasting plasma glucose that required immediate drug therapy according to national guidelines (Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, Adult Treatment Panel II, and American Diabetes Association).

We excluded those persons who planned to move from the area during the trial; who were already taking lipid-altering medications (inclusive of niacin); who smoked, consumed alcoholic beverages exceeding an average of 3 drinks/d, or consumed coffee >710 mL/d; and women who were pregnant or lactating. We accepted postmenopausal women whether or not they were taking hormone replacement therapy and those persons currently receiving standard medical therapy (for conditions such as hypertension, arthritis, or other chronic diseases) if they agreed to continue with their current therapy during the trial. If participants were unsure of their cholesterol concentration before baseline screening but thought it was high, we informed them of their baseline cholesterol concentration so that they could make an informed decision whether to enter the trial or seek medical counsel. We advised those participants with LDL cholesterol >4.14 mmol/L of the risks associated with LDL-cholesterol concentrations of this nature and recommended that they see their physician. However, we allowed those participants who wanted to continue in the study to do so.

Inclusion criteria and pretrial screening

We used a 4-phase approach to perform our trial. These phases included a telephone screening procedure to determine potential eligibility; 3 baseline visits, including 2 run-in screening visits; 6 wk of treatment, including a midstudy visit at 3 wk; and a posttest assessment. A general schematic of our trial is presented in **Figure 1**. During phase 1, we screened 500 potential participants by phone. This screening resulted in 250 persons who qualified to begin the baseline testing portion of our trial. Once participants were found to be eligible to continue the trial, we asked each participant to partake in 3 baseline visits during the second phase of our trial before formal random assignment into the trial. During the first 2 visits of this phase of testing, we had each participant make 2 run-in visits within 7 d of each other. Successful continuation to the third baseline visit was predicated on obtaining 2 TC concentrations that differed by ≤10%. If the TC difference between the 2 run-in visits was >10%, we added a third run-in visit and we accepted the average of the 2 closest baseline measures for the entry criterion lipid variable. After this run-in procedure, 90 participants remained eligible to continue to the

third baseline visit of the trial, when we drew a larger volume of blood to examine other blood markers, including a basic chemistry panel (Chem-16) to screen for potential side effects.

In conjunction with the third baseline visit, each participant was required to fill out several questionnaires that detailed his or her medical history and dietary habits. These questionnaires were made available by an Internet data-collection system (Vital Link, Bellevue, WA). We mailed questionnaires to those participants without Internet access. Once participants completed all of these forms and all 3 baseline visits, they became eligible for formal random assignment into the treatment portion of the trial. If these conditions were not met, the participant was not randomly assigned into the treatment portion of the study. The time elapsed between the first run-in visit and the third baseline collection period ranged from 10 to 12 d.

The rationale for using this type of enrollment procedure was to establish stable baseline entry criteria for TC and to attempt to minimize dropouts and maximize protocol compliance by dissuading from continuing in the study those persons who were less likely to complete the trial given their low willingness to adhere to the study procedure. Of the 90 participants eligible for random assignment, 10 withdrew before random assignment because of a “lack of continued interest” in pursuing the study, and 24 failed to complete all baseline visits, necessary paperwork, or both. Thus, 56 participants received formal random assignment to undergo treatment and enter the third phase of the study, ie, the treatment phase. During this treatment phase, all participants agreed to maintain their current dietary intake and exercise habits and to avoid beginning the consumption of any new supplements or medications. Given that we studied a free-living population and recognized that alterations in body mass and energy intake can affect cholesterol concentrations, we also weighed participants and collected food-frequency questionnaires before (baseline) and after (6 wk) intervention to examine total energy intake and macronutrient partitioning.

To account for dietary intake, we used the FOOD INTAKE ANALYSIS SYSTEM (FIAS; version 3.9, Human Nutrition Center, University of Texas Health Science Center School of Public Health, San Antonio). One reason we selected FIAS is that it is linked with the Pyramid Serving Database. The USDA food codes generated after the analysis of the dietary recalls in FIAS are linked to the Pyramid Serving Database to determine the number of servings of each major food group consumed. This database was developed to analyze the number of servings of each of the major food groups of the food guide pyramid and the amounts of discretionary fat and sugars consumed (15–17).

Treatment and assessment indexes

We randomly assigned each participant in a double-blind manner to consume 10 capsules of bIg or placebo per day. Each capsule contained 500 mg of the appropriate treatment. Participants then proceeded to ingest 5 capsules, twice a day, for 6 wk. The 10-capsule treatment regimen provided 5 g bovine immunoglobulin G daily or 2.4 g bIg. We verbally instructed participants to take their respective supplements with a meal. We chose this dose for 2 reasons. First, in-house pilot data showed it to be efficacious, and we subsequently decided on the sample size for this trial on the basis of power calculations obtained from this pilot effort. Second, because we used capsules, the possibility of using a larger dosage was prohibitive.

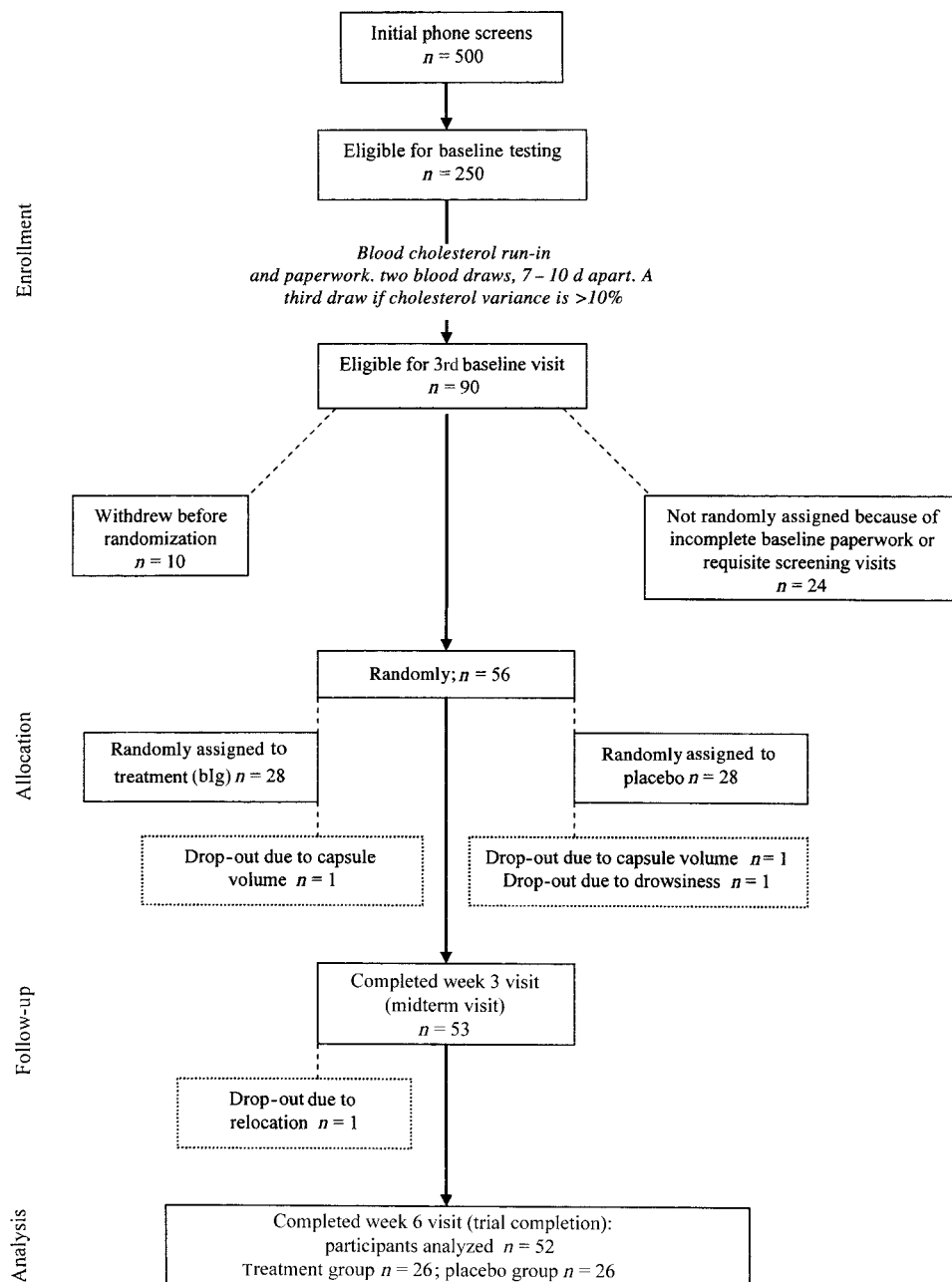


FIGURE 1. Schematic representation of study timeline. bIg, bovine serum immunoglobulin.

The source of bIg used in this study is an immunoglobulin-enriched serum protein fraction obtained through the partial removal of fibrinogen, lipids, and albumin. The resulting total protein fraction was 88.6%, and the remainder of the chemical constituents were moisture (<8%) and ash (<3%). The total immunoglobulin G was 48.4% as determined by radial immunodiffusion assay. We used hydrolyzed gelatin as the source of protein for the placebo group. All treatment bottles and capsules were identical in size and color; they were distributed by a randomized number sequence for treatment distribution so that, in the case of side effects, we could break the treatment code by subject rather than by group. One of the investigators (ANJ) was in charge of this aspect of the study and had no contact with the study participants at any time and was not involved in the data

analysis. On entry into the trial, we gave participants enough bIg or placebo for the first 3 wk of the trial. All participants returned at 3 wk for an interim blood measurement; at that time, we also collected treatment bottles to perform a capsule count to determine compliance. We then gave participants enough bIg or placebo for the second 3 wk of the trial, ie, enough capsules to complete the trial. When all participants returned at 6 wk, we collected treatment bottles again to perform another capsule count.

We obtained all blood assessments in a fasting condition (>12 h) for a variety of variables including a blood lipid profile, blood glucose concentration, and muscle, kidney, and hepatorenal indexes (Chem-16). Each blood collection consisted of obtaining ≈ 50 mL blood divided into 1 serum separator evacuated tube (10

mL) and 4 K3 EDTA-coated tubes (≈ 40 mL). All blood samples were spun within 3 min of venous collection in a cold centrifuge at $2383 \times g$ for 15 min at 4°C . Separated plasma and red blood cells were divided into 4 cryovials and placed in -80°C freezers.

Blood lipid profiles, fasting blood glucose concentration, and muscle and hepatorenal indexes were analyzed at a commercial laboratory (LabCorp, Dallas, TX). We used the formula of Friedewald et al (18) to calculate LDL cholesterol, in which $\text{LDL cholesterol} = [\text{TC} - (\text{HDL cholesterol} + \text{triacylglycerol}/5)]$. To validate the use of an external laboratory, where minor differences in cholesterol may be related to the time and day of the analysis, we randomly chose 40 frozen blood samples for a reliability analysis in a different laboratory. The results of this secondary analysis showed no difference for TC or LDL cholesterol with the data obtained from LabCorp ($\text{CV} = 0.08$; $r^2 = 0.96$).

Statistical analysis

The primary variables that we examined during this trial were TC and LDL cholesterol. All other lipid subfractions inclusive of VLDL cholesterol, HDL cholesterol, and triacylglycerols were treated as secondary outcome measurements. As tertiary measures, we examined blood glucose and the Chem-16 panel. We examined all baseline and all primary and secondary outcome measurements for normal distribution characteristics. As such, each variable was found to be normally distributed; thus, we did not adjust our data. To examine potential treatment differences in our study, we examined all primary, secondary, and tertiary variables separately with the use of a repeated measures multivariate analysis of covariance (MANCOVA), covaried for a change in total energy expenditure and alcohol intake for both groups with the use of a 3-step approach. First, we performed our initial MANCOVA to determine the overall time effects and treatment time \times treatment interaction for statistical significance. Second, if we observed a significant statistical or interaction effect for the overall MANCOVA, we evaluated the changes in individual lipid variables by examining the treatment effect at each time point (ie, baseline, 3 wk, and 6 wk). Third, we used a Tukey-Kramer post hoc test to examine appropriate within- or between-group changes at 3 wk and 6 wk. We assessed all food-frequency data with a 2×2 analysis of variance to denote before and after and between- and within-group food values. All statistical analyses were performed with the use of JMP statistical software (version 5.0.1.2; SAS Inc, Cary, NC). Statistical significance refers to a P value of <0.05 . All data are reported as mean \pm SD.

RESULTS

After formal random assignment to the treatment phase of our protocol ($n = 56$), 4 participants dropped out of the study. These persons were equally distributed between the placebo and bIg groups. Three of these persons (2 and 1 in the placebo and bIg groups, respectively) dropped out before the 3-wk visit. Two of these dropouts cited the number of capsules to be taken daily as the reason for stopping the study. One participant asked to drop out because "the treatment made her feel drowsy." However, after breaking her treatment code, we determined that she was in the placebo group. The participant in the bIg group continuing past the 3-wk visit dropped out because of an unexpected job move to a different state. Because 3 of the randomly assigned

participants did not attend the 3-wk visit, we elected not to perform an intent-to-treat analysis. We based this decision on the observation that including the 3 subjects who initiated baseline testing and did not attend the 3-wk visit and the 1 participant who made the 3-wk visit did not change the outcome of the study or any of the data reported below.

We found no differences for age, BMI, or any lipid variable at baseline. The mean (\pm SD) age for our participants was 51 ± 8.2 y. Baseline body mass was 79.96 ± 10.2 kg. The bIg group consisted of 16 men and 10 women. The placebo group consisted of 15 men and 11 women. We observed no gender interactions for any of the subsequent treatment analyses. Throughout the study, each group remained $\approx 75\%$ compliant with the study protocol, and the number of capsules for daily ingestion was cited as the primary reason for lack of compliance. We observed no difference in compliance between the bIg (78%) and placebo (73%) groups ($P = 0.33$). However, we did observe a significant main effect of time ($P < 0.01$) on total energy consumption during the study period. Further analysis of the individual macronutrients found no other significant effects. These macronutrients included carbohydrates, protein, total fat, and associated subfractions of fat (saturated, polyunsaturated, or monounsaturated fat), dietary cholesterol, and dietary fiber (**Table 1**). We also observed a significant main effect of time with regard to alcohol intake ($P < 0.01$). Because all of these variables are collinear in some fashion with total energy consumption, and also affect cholesterol metabolism, we used changes in total energy consumption and alcohol intake as covariants in our analysis. Furthermore, because emerging research has shown a relation between body weight and milk intake (19, 20) to be a potential influence on lipid outcomes, we also examined milk intake and found no differences between or within groups for total milk intake, low-fat milk intake ($<2\%$ fat), and high-fat milk intake ($\geq 2\%$ fat). On average, total milk intake ranged between 0.20 and 0.43 servings (227 mL each)/d for both the placebo and bIg groups.

Primary lipid variables

With the repeated-measure MANCOVA, we observed significant overall main effects of time ($P < 0.001$) and treatment ($P < 0.03$) and a significant time \times treatment interaction ($P < 0.0001$). Subsequently, we examined each lipid variable individually and found both TC and LDL cholesterol to be statistically significant for time and treatment at 3 and 6 wk (both: $P < 0.0001$). We also observed a significant between-group difference in TC ($P < 0.005$) and LDL cholesterol ($P < 0.007$) at 6 wk. On the basis of these results, we performed Tukey's post hoc assessment of TC and LDL cholesterol for the within-group (ie, time) effects observed at 3 and 6 wk. However, we examined only the between-group effects at 6 wk because no significant group effect was noted at 3 wk. The results of this analysis are fully detailed in **Table 2**.

Overall, our post hoc assessment showed a significant within-treatment group reduction in TC from baseline to 3 wk (-0.35 ± 0.5 mmol/L, $P < 0.05$) and 6 wk (-0.36 ± 0.6 mmol/L, $P < 0.05$). By 6 wk, the reduction in TC from baseline in the treatment group was significantly different from that in the placebo group ($P < 0.05$). We observed no significant within-group difference from baseline in the placebo group at 3 wk (-0.03 ± 0.5 mmol/L) or 6 wk (-0.10 ± 0.4 mmol/L). The reduction we observed in TC was due to a significant reduction in the LDL-cholesterol subfraction: we found a significant within-treatment

TABLE 1Nutrient characteristics for the study cohort during the experimental period¹

Nutrient intake	Bovine serum immunoglobulin (bIg) group (n = 26)		Placebo group (n = 26)	
	Baseline	Week 6	Baseline	Week 6
Total energy (kJ · d ⁻¹) ²	7862.00 ± 3472.4	6814 ± 1968.2	7644.00 ± 2784.1	6459 ± 3548.9
Protein (g · d ⁻¹)	77.70 ± 41.3	70.50 ± 47.4	84.40 ± 62.2	62.20 ± 40.8
Carbohydrates (g · d ⁻¹)	223.80 ± 110.6	199.70 ± 96.9	235.60 ± 146.3	195.30 ± 133.6
Fat intake (g · d ⁻¹)				
Total fat	70.70 ± 38.8	67.20 ± 50.5	74.80 ± 55.1	56.90 ± 38.8
Saturated fat	23.20 ± 12.7	21.60 ± 16.8	25.00 ± 20.4	18.60 ± 12.7
MUFA	26.80 ± 14.8	25.90 ± 19.4	28.40 ± 21.4	21.50 ± 14.8
PUFA	15.10 ± 8.7	14.60 ± 10.2	15.00 ± 10.2	12.10 ± 8.7
Diet cholesterol (mg · d ⁻¹)	264.30 ± 189.2	221.70 ± 158.1	273.40 ± 262.1	193.20 ± 139.7
Alcohol (g · d ⁻¹) ²	10.50 ± 27.5	4.91 ± 7.1	8.10 ± 12.2	5.91 ± 7.6
Fiber (g · d ⁻¹)	18.10 ± 8.7	16.40 ± 7.6	20.10 ± 13.8	16.10 ± 12.7

¹ All values are $\bar{x} \pm$ SD. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.² Significant main effect for time, $P < 0.001$ (ANOVA). There were no other significant main effects or interactions.

group reduction from baseline in LDL cholesterol at 3 wk (-0.20 ± 0.3 mmol/L, $P < 0.05$) and 6 wk (-0.28 ± 0.4 mmol/L, $P < 0.05$). By 6 wk, we observed a significant difference between groups: Tukey's post hoc examinations showed LDL cholesterol to be significantly lower in the bIg group than in the placebo group ($P < 0.05$). No within-group treatment differences from baseline were observed in the placebo group at 3 wk (0.05 ± 0.4 mmol/L) or 6 wk (-0.03 ± 0.4).

Secondary lipid variables and tertiary outcomes

As it pertains to our secondary lipid analysis (ie, HDL cholesterol, VLDL cholesterol, and triacylglycerol), we found significant main effects of time ($P < 0.001$) and treatment ($P < 0.0001$) and a significant time \times treatment interaction ($P < 0.001$). Despite this overall MANCOVA result, we found no other significant statistical results for the marker estimates associated with HDL cholesterol, VLDL cholesterol, triacylglycerols, or glucose. Furthermore, we did not observe any significant changes in other blood chemistry markers associated with hepatorenal or cardiovascular function.

DISCUSSION

In this trial, we found a significant reduction in TC and LDL cholesterol accompanying bIg supplementation in participants with hypercholesterolemia. The reduction in LDL cholesterol is an encouraging finding because consumers can ingest bIg in capsules or by incorporation into food products. Another redeeming feature of bIg is that persons can consume relatively low doses, which allows them to follow dietary guidelines from the National Cholesterol Education Program Step II and to make other dietary changes without an excessive additional calorie intake. Although we do not know the exact mechanism of action for bIg's lipid-lowering effect, previous research findings suggest several potential mechanisms, including a hypocholesterolemic effect of milk or fermented milk, the lipogenic effects of endotoxin and cytokines (21), and factors in plasma, such as plasma apolipoproteins, that may affect cholesterol absorption (14, 22).

The potential hypocholesterolemic effect of milk and fermented milk products was initially proposed from a study that

involved the Masai people of Africa, whose normal diet is atherogenic and consists of large amounts of meat, milk, and blood serum (23). In that report, the consumption of up to 8 L milk/d resulted in lower TC, despite weight gain in the participants.

TABLE 2Serum lipid characteristics of the groups during the experimental period¹

	Bovine serum immunoglobulin (bIg) group (n = 26)	Placebo group (n = 26)
	Total cholesterol (mmol/L) ²⁻⁴	
Baseline	6.33 ± 0.1	6.16 ± 0.1
3-week ³	5.98 ± 0.5 ⁵	6.13 ± 0.6
6-week ^{3,4}	5.97 ± 0.7 ^{5,6}	6.06 ± 0.5
LDL cholesterol (mmol/L) ²		
Baseline	4.12 ± 0.6	3.95 ± 0.5
3-week ³	3.92 ± 0.7 ⁵	4.00 ± 0.6
6-week ^{3,7}	3.84 ± 0.6 ^{5,6}	3.92 ± 0.6
HDL cholesterol (mmol/L)		
Baseline	1.35 ± 0.05	1.49 ± 0.04
3-week	1.28 ± 0.3	1.46 ± 0.3
6-week	1.29 ± 0.3	1.52 ± 0.3
VLDL cholesterol (mmol/L)		
Baseline	0.87 ± 0.5	0.71 ± 0.3
3-week	0.78 ± 0.4	0.73 ± 0.3
6-week	0.84 ± 0.5	0.62 ± 0.2
Triacylglycerol (mmol/L)		
Baseline	1.84 ± 0.9	1.51 ± 0.6
3-week change	1.65 ± 0.8	1.43 ± 0.7
6-week change	1.77 ± 1.0	1.35 ± 0.8

¹ All values are $\bar{x} \pm$ SD.² Significant overall main effects of time ($P < 0.001$) and treatment ($P < 0.03$) and significant time \times treatment interaction ($P < 0.0001$) (repeated-measures multivariate analysis of covariance).³ Statistically significant for time and treatment (both: $P < 0.0001$).^{4,7} Statistically significant for time \times treatment (ie, between-group) interaction: ⁴ $P < 0.005$, ⁷ $P < 0.007$ ⁵ Significant within-group (ie, time) difference from baseline, $P < 0.05$ (Tukey-Kramer post hoc analysis).⁶ Significantly different from placebo group, $P < 0.05$ (Tukey-Kramer post hoc analysis).


Several other clinical studies on milk and fermented milk consumption appear to confirm these findings (4, 6, 8, 11, 24–28). Moreover, one study that compared soy protein with or without isoflavones with milk protein reported an unexpected reduction in LDL cholesterol in participants who consumed 42 g milk protein/d (29). Still, the mediating factors promoting a reduction in cholesterol remain elusive, although immunoglobulin G (11), magnesium (30), riboflavin (30), orotic acid (30), increased bacterial activity (31), and an unknown inhibitory factor (23) have been proposed as potential mediators. The principal constituents of milk that can be eliminated as potential cholesterol-lowering mediators, given their hypercholesterolemic nature, include saturated fat, lactose, calcium, and casein (10). A factor that complicates the comparison of our study with others is the fact that the globulin protein concentration of previous studies involving milk products is unknown.

In our trial, we used a product with a high bIg concentration, which showed a pronounced lipid-lowering effect. This finding is in agreement with previous observations that used an immune milk product (12). It is notable that the TC at baseline for our participants was substantially lower (6.33 mmol/L) than that in the 2 previous studies that used skim milk from immunized cows [6.86 mmol/L (12)] and yogurt and whole milk [7.26 mmol/L (4)]. Despite the lower baseline TC in our present study, supplementation with 5 g bIg resulted in a similar reduction in TC concentration as were seen with the supplementation with 90 g skim milk solids in the previous study of immune milk. A point that should also be considered is whether the effects of bIg supplementation reported in this study point to a means of modulating circulating cholesterol different from previous dietary approaches, such as the use of phytosterols, soluble fiber, or soy protein, which affect the absorption of cholesterol.

Data on the effects of feeding plasma protein components on cholesterol metabolism are scarce. The only previously reported animal study to evaluate the effects of plasma protein components on cholesterol absorption or metabolism found no differences in serum or liver cholesterol concentrations between animals fed soy protein and those fed plasma protein, whereas significant increases in liver cholesterol concentration were reported in those fed casein (9). However, bIg is not absorbed in healthy adults, so the effects are most likely luminal. A luminal effect is possible because the gut is an important contributor to circulating cholesterol, second only to the liver in the body (32, 33). In addition, endotoxin, a proinflammatory molecule with a presence in the lumen, has been shown to be lipogenic and is proposed to be a contributor to atherosclerosis and cardiovascular disease because of its proinflammatory effects (34).

Endotoxin-mediated lipogenesis has been found in animal models to be mediated through increased 3-hydroxy-3-methylglutaryl-coenzyme A reductase and reduced cholesterol 7- α -hydroxylase activity (35–38). It is interesting that immunoglobulin and other plasma proteins possess an endotoxin-binding capacity that, when they are administered orally in sufficient quantity, has the potential to bind endotoxin in the lumen and subsequently affect inflammatory cytokine production in the intestine (39). Helping to substantiate this premise is the finding that the oral administration of an immunoglobulin A–bIg fraction of human plasma affects inflammatory cytokine production (40). In addition, bovine plasma contains relatively high concentrations of HDL and LDL (41). HDL, in particular, is known to bind endotoxin, which may indirectly reduce the stimulation of

cholesterol synthesis by mediation of inflammatory cytokines (42, 43). Both HDL and LDL were also shown to directly inhibit cholesterol synthesis *in vitro* (44). Antibodies and other plasma proteins found in normal human and animal plasma may also be reducers (45, 46). These antibodies may directly bind cholesterol in the lumen and hinder absorption. In addition, apolipoprotein A-I, obtained from sheep plasma, was shown to reduce the brush-border uptake of cholesterol through inhibition of a scavenger-type receptor (14, 22).

Although many studies have evaluated the effects of dietary protein sources on cholesterol concentrations, few studies evaluated specific animal protein components, and no clinical studies about the effects of plasma protein fractions on cholesterol metabolism have been conducted. Although our results are encouraging, treatment compliance may have limited our potential finding of a more pronounced effect. Limited compliance was likely due to the requirement that participants ingest 10 capsules daily. Thus, an alternate means of bIg administration, such as a beverage or a snack or food bar, should be considered for future studies. In addition, future studies should examine the optimal amount of bIg supplementation relative to differing LDL-cholesterol subfractions. Nonetheless, this study shows that globulin protein supplementation reduces TC and LDL cholesterol in a population with mild hypercholesterolemia. In view of the hypercholesterolemia problem in industrialized countries, these results should encourage further studies examining the effects of globulin protein supplementation on cholesterol metabolism in humans. 

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CPE served as primary investigator for this study and was responsible for all final statistical analyses and for writing the manuscript. ANJ orchestrated the organization, coding, and assignment of all treatments, as well as assisting in the writing of this paper. MS assisted in participant recruitment and manuscript preparation. EW assisted in the study design, data analysis, and writing of the manuscript. TSC served as medical director for the project and assisted in study design and manuscript preparation. EW is vice president of Proliant Inc and in charge of scientific research and development. In this role, he has overseen many animal trials and was directly responsible for bringing this trial to clinical population. EW's role in this study was limited to intellectual and design functions, and he did not partake in any of the clinical aspects of the trials. None of the other authors had any personal or financial conflicts of interest.

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