The whey fermentation product malleable protein matrix decreases triglyceride concentrations in subjects with hypercholesterolemia: A randomized placebo-controlled trial¹

H. K. Berthold,^{*2} D. M. Schulte,[†] J.-F. Lapointe,[‡] P. Lemieux,[‡] W. Krone,[†] and I. Gouni-Berthold[†]

^{*}Lipid Clinic at the Interdisciplinary Metabolism Center and Department of Geriatrics, Charité University Medicine, 13353 Berlin, Germany

[†]Department of Internal Medicine II, University of Cologne, 50937 Cologne, Germany

[‡]Technologie Biolactis Inc., Laval, QC, H7V 5B7, Canada

ABSTRACT

Malleable protein matrix (MPM) is a unique whey-derived ingredient obtained through a fermentation process using proprietary lactic acid bacteria strains from the *Lactobacillus kefirifaciens* species. Because evidence from animal models suggests that MPM decreases serum lipid concentrations, the purpose of the present trial was to assess the hypothesis that MPM exerts lipid-lowering effects in humans. A total of 161 subjects (50% male; age 54.5 ± 9.8 yr, body mass index 26.3 ± 3.6 kg/m²) with hypercholesterolemia with baseline low-density lipoprotein cholesterol (LDL-C) levels of 181 ± 30 mg/dL and normal triglyceride (TG) levels (131 ± 55 mg/dL) were randomized to receive MPM (2×15 g/d) or matching placebo. A 6-wk run-in phase was followed by a double-blind 12-wk treatment phase after randomization. The data were analyzed on an intention-to-treat basis. The primary outcome measure was the percentage change of LDL-C. The secondary outcome measures were changes in TG and high-density lipoprotein cholesterol concentrations as well as changes in other cardiovascular risk factors. After 12 wk of treatment, the relative TG decrease from baseline reached 9.8%, whereas LDL-C was slightly decreased (by 1.5%) following MPM treatment compared with placebo in the intention-to-treat cohort. The treatment effect on TG reduction was much higher in the subset of subjects having TG levels at baseline of 150 mg/dL or above ($n = 42$), reaching 20.0% compared with placebo. High-density lipoprotein cholesterol concentrations, blood pressure, and fasting blood glucose remained unchanged, whereas a positive treatment effect was seen on hemoglobin A_{1c}. The MPM product was tolerated well without severe adverse events. In conclusion, MPM

has significant TG-lowering properties in subjects with combined hypercholesterolemia and higher TG levels. Its effects on LDL-C concentrations and glucose metabolism deserve further investigation.

Key words: whey, malleable protein matrix, hypercholesterolemia, hypertriglyceridemia

INTRODUCTION

Hypercholesterolemia, especially an elevated concentration of low-density lipoprotein cholesterol (LDL-C), is a major risk factor for coronary heart disease (CHD). Although statins are the first-line drugs recommended in the third report of the National Cholesterol Education Program (NCEP) to treat nonoptimal LDL-C (NCEP ATP-III, 2001), a significant proportion of subjects with moderate hypercholesterolemia either do not want to initiate or cannot tolerate drug treatment, or otherwise cannot achieve their treatment goals for LDL-C. For these subjects, strategies combining diets, physical activities, and “natural” options to lower cholesterol may increase compliance and offer an acceptable alternative or addition to the armamentarium of lipid-lowering agents.

Under controlled conditions, diets recommended by the American Heart Association (AHA) and NCEP have been shown to decrease LDL-C by 10 to 20%. However, in outpatient, free-living settings, less pronounced improvements with diet alone have been observed, with reductions of around 5% usually reported (NCEP ATP-III, 2001; Jenkins et al., 2002). Besides restricting the intake of macronutrients such as saturated fats and cholesterol, the introduction of therapeutic dietary nutrients or foods in the diet that are able to lower LDL-C and improve other CHD risk factors is gaining a prominent position in dietary guidelines and recommendations (Lichtenstein and Deckelbaum, 2001; Lichtenstein et al., 2006; Rudkowska and Jones, 2007; Rudkowska, 2008, 2009). Among these dietary options, the protein fraction of whey, a fluid derived from milk, has been shown to have a variety of beneficial properties such as

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²Corresponding author: heiner.berthold@charite.de

immune-enhancing, antioxidant, antihypertensive, and lipid-lowering effects (Marshall, 2004).

The end product called malleable protein matrix (MPM) is obtained by concentration of constituents with a centrifugation step following whey fermentation using *Lactobacillus kefiranoformans*. Before pasteurization, this procedure enables the enrichment of MPM with selected whey proteins, calcium, various fermentation metabolites (i.e., exopolysaccharides), and *Lb. kefiranoformans*. In the final MPM, high-molecular-weight whey proteins such as lactoferrin and immunoglobulin become highly concentrated compared with levels in the initial fluid whey (Lapointe et al., 2009). The MPM product has been classified as a whey protein hydrolysate by the Chemical Abstract Service and given the number 308074-13-7. To date, numerous animal studies have been performed with MPM and have demonstrated systemic antiinflammatory and immunomodulating effects (Beaulieu et al., 2007a,b, 2009a) as well as triglyceride (TG) and LDL-C-lowering properties (Beaulieu et al., 2010).

The present trial (European Clinical Trials Database EudraCT no. 2006-006738-16) was performed to investigate, for the first time, potential lipid-lowering effects of MPM and its tolerability in humans. The primary objective was to show a decrease in LDL-C compared with placebo in subjects with primary hypercholesterolemia. In addition, its abilities in beneficially affecting other lipid parameters such as TG as well as other CHD risk factors associated with glucose metabolism, blood pressure (BP), and various anthropometric data were investigated as secondary outcome measures.

MATERIALS AND METHODS

Eligible Subjects

Female and male subjects aged 18 yr or older with isolated hypercholesterolemia or combined hyperlipidemia [LDL-C levels of ≥ 130 and < 190 mg/dL (≥ 3.3 and < 4.9 mmol/L)] were eligible for enrollment. Major exclusion criteria included TG levels ≥ 400 mg/dL (≥ 4.5 mmol/L), body mass index (BMI) > 35 kg/m², a history of cardiovascular disease (defined as known CHD, stroke, or peripheral arterial occlusive disease), diabetes mellitus, severe gastrointestinal or liver disease, major surgery < 8 wk before the study, untreated or clinically evident thyroid disease, drug or alcohol abuse, significant weight loss ($> 10\%$) in the last 6 mo before the study, intolerance or allergies to lactose or milk proteins, concomitant treatment with statins or other lipid-lowering drugs (including weight loss drugs and fat absorption inhibitors), or intake of any drug or

supplement known to affect lipid metabolism during a period of < 12 wk before study entry.

Study Design and Protocol

The study was a multi-center, double-blind, randomized, placebo-controlled trial with 2 parallel treatment groups, which was conducted between July 2007 and April 2008. The participating centers included 1 university hospital outpatient lipid clinic, 1 university sports medicine department, and 7 general medical practices. The trial was performed according to the latest version of the Declaration of Helsinki, taking into account the current version of German drug legislation and it was in accordance with the International Conference on Harmonization guideline for Good Clinical Practice CPMP/ICH/135/95. The study protocol was approved by the ethics committee of the University of Bonn and other competent ethics committees. Written informed consent was obtained from all participants.

The study design and flowchart of the trial are shown in Figure 1. During the screening visit, eligible subjects were given diet and lifestyle recommendations according to the 2006 AHA nutrition committee scientific statement (Lichtenstein et al., 2006). A leaflet titled "10 Guidelines for a Wholesome Diet" published by the German Nutrition Society was handed to each eligible subject. Subjects were asked to implement, as possible, these recommendations and maintain them throughout the study. Dietary counseling was reinforced at each subsequent visit and a 3-d food diary was collected before randomization (visit 3) and at the final visit (visit 6). Following a 6-wk run-in phase, subjects whose LDL-C had not normalized below 130 mg/dL were randomized to receive either 2×15 g/d of MPM or placebo in a double-blind manner. The randomization was stratified by sex and center. The nutritional composition of MPM and placebo is shown in Table 1. The trial product doses were supplied as a ready-to-mix powder juice formula by Technologie Biolactis Inc. (Laval, QC, Canada) in individual sachets. Each sachet had to be dissolved in 250 mL of water using a blender and provided 15 g of MPM or a placebo matching for protein (gelatin), carbohydrates, and calcium. Study participants had to take 2 sachets daily with meals (morning and evening). The product, once prepared, was stable for 12 h at 4°C. Placebo was to be prepared in the exact same way.

Blood was drawn after an overnight fast for lipid and lipoprotein analysis, other laboratory parameters, and safety parameters at the screening visit 1 (-6 wk), visit 2, and visit 3 (on separate days in wk -1), visit 3 (d 0), visits 4, 5, and 6 (3, 6, and 12 wk after d 0, respectively; see Figure 1).

Figure 1. Study design and visit schedule of the double-blind, randomized, placebo-controlled trial. Following a 6-wk run-in, 3 visits (visit 4, 3 wk; visit 5, 6 wk; visit 6, 12 wk) were planned during treatment to assess the change from baseline in efficacy variables. Treatment = malleable protein matrix (MPM) 15 g or matching placebo twice daily during 12 wk (84 d). NCEP = National Cholesterol Education Program.

Outcome Variables

The primary objective of this trial was to show the LDL-C decrease by MPM compared with placebo. Secondary efficacy variables were changes in TG, total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) as well as derived cholesterol ratios and fractions, apolipoprotein-B (Apo-B), fasting glucose (GLU) and insulin (INS), homeostatic model assessment (HOMA) index ($GLU \times INS$), glycated hemoglobin A_{1c} (HbA_{1c}), high-sensitivity C-reactive protein (CRP), BW, BMI, waist and hip circumference, waist-to-hip ratio, and systolic (sBP) and diastolic (dBP) BP.

Safety and Tolerability Assessments

The safety profile and tolerability were evaluated by recording adverse events (AE) as well as measurement of vital signs, chemistry, and hematology variables at

each clinical visit. The AE documented by the investigators were categorized according to severity (mild, moderate, and severe) and causality to the study products (unrelated, unlikely, possible, probable, certain). Adverse events were coded according to the World Health Organization-Adverse Reaction Terminology (World Health Organization Uppsala Monitoring Center, 2010). The frequencies and incidence rates were calculated on a per patient basis and analyzed according to O'Neill (1987).

Laboratory Methods and Clinical Assessments

Commercially available kits and reagents were used to determine TC, HDL-C, TG (CHOD-PAP and GPO-PAP enzyme assays), GLU (hexokinase/G6PDH method) and CRP (immunoturbidimetric latex highly sensitive assay) using an Olympus 2700 analyzer (Olympus, Hamburg, Germany). Low-density lipoprotein chole-

Table 1. Composition of malleable protein matrix (MPM) and placebo (per 100 g dry basis) and corresponding daily dosage¹ of the trial products

Component	MPM		Placebo	
	Per 100 g	Per daily dosage	Per 100 g	Per daily dosage
Calories	296.0	103.0	276.0	93.0
Proteins, g	33.4	11.6	34.3	11.6
Carbohydrates, g	33.1	11.6	33.8	11.4
Lactose, g	11.5	4.0	23.2	7.8
Fat, g	3.0	1.0	0.3	0.1
Calcium, g	7.3	2.6	7.3	2.4
Sodium, g	0.7	0.3	0.0	0.0

¹Composition corresponding to a daily dosage of 2 sachets containing the ready-to-mix powder formula of MPM or placebo.

terol was determined according to the Friedewald equation [TC – HDL-C – (TG/5)]; and INS determination (enzymeimmunoassay) was performed on an Immulite 2000 analyzer (Siemens, Eschborn, Germany). Apolipoprotein-B determination (nephelometric assay) was performed using a BN II system (Siemens, Eschborn, Germany). Finally, HbA_{1c} was determined from EDTA whole blood by HPLC. The HOMA index was calculated according to the following formula: $\text{INS } [\mu\text{U/mL}] \times \text{GLU } [\text{mmol/L}] / 22.5$ or $\text{INS } [\mu\text{U/mL}] \times \text{GLU } [\text{mg/dL}] / 405$. Biochemical and hematologic safety parameters were determined by standard laboratory methods. All analyses were carried out in a central laboratory that was certified according to the standards of Good Laboratory Practice.

The sBP and dBP were determined at every visit using the same blood pressure cuff on the same arm and if possible at the same time of the day after at least 5 min at rest in sitting position. The heart rate was measured at rest in a sitting position.

The waist circumference was taken with a flexible tape (held horizontally) at the smallest circumference between the lowest part of the costal arch and the crista iliaca of the hip bone while study participants were freestanding. The hip circumference was taken with a flexible tape (held horizontally) at the widest part of the buttocks between the hip bone [crista iliaca] and the crotch while participants were in a freestanding position.

Statistical Analysis

The intention-to-treat population (ITT) defined for the efficacy analyses included all randomized subjects who received at least one dose of the trial product, including placebo, and for whom at least one observation visit under medication was available regardless of study protocol violations. The per-protocol population (PP) was restricted to subjects who did not have any major study protocol violations. The PP was the secondary population for the efficacy analyses. Protocol violations were characterized (minor or major) under blinded conditions. Safety and tolerability were assessed for all subjects who had taken at least one dose of the trial product, including placebo.

Treatment adherence was calculated at each visit during the treatment phase based on used and unused trial products returned by the study participants from their previous visit. Subjects were classified as adherent if they had taken at least 80% but not more than 120% of the trial products. A $\pm 10\%$ adherence deviation was rated as a minor protocol deviation.

The primary efficacy parameter was the percentage change of LDL-C from pooled baseline (mean value of

visits 2 and 3) to study end point (visit 6 for completers and last visit available under study medication for drop-outs). This was tested initially by means of a parametric ANOVA with effects due to treatment, sex, center, and the treatment-by-center interaction. Homogeneity of treatment effect across centers was checked with prespecified pooling criteria defined by either center size (small centers combined) or medical setting (outpatient clinic versus other practitioners combined). The analysis of all other secondary variables at study endpoint or at each intermediate visit was done with the same model. Finally, the effect of treatment across study visits was explored by ways of repeated-measure ANOVA.

Sample size estimation was based on the percentage change of LDL cholesterol from pooled baseline, a level of significance of $P < 0.05$ (2-sided alternative), and a power of 80%. It was expected that the active treatment would result in an LDL cholesterol decrease of $\geq 10\%$ (with a standard deviation of 20%) compared with placebo. Thus, an effect size (Cohen's d) of $10/20 = 0.5$ was expected. To achieve the calculated power, 64 evaluable patients were needed in each treatment group. We planned for a total of 75 patients to be enrolled per treatment group because of an expected drop-out rate of about 17%.

The statistical package SAS version 8.02 (SAS Institute Inc., Cary, NC) was used for general calculations. Sample size was calculated by using the software "Nquery" version 4.0 (Statistical Solutions, Cork, Ireland). Diet protocols were evaluated by using Prodi version 5 software (Wissenschaftliche Verlagsgesellschaft Stuttgart, Germany). Changes from baseline to last visit were calculated for total calories, fat (total, saturated, monounsaturated, and polyunsaturated fatty acids), protein, carbohydrates, alcohol, total cholesterol, and fiber intake. These variables were compared between the 2 treatment arms by means of a one-way ANOVA.

RESULTS

Subject Disposition and Demographic and Baseline Characteristics

The flow of participants through the trial is presented in Figure 2. A total of 222 subjects were enrolled in 9 trial centers, of which 161 subjects were randomized into the double-blind treatment phase and 154 included in the ITT cohort for efficacy analyses. Of the randomized participants, 81 were assigned to the MPM treatment and 80 to the placebo. A total of 27 subjects (16.8%) terminated the trial prematurely. The most common reasons were "adverse events" (7.5%) and "withdrawal of consent" (3.7%). A total of 131 subjects completed

Figure 2. Flow of participants through the trial. Excluded participants from the per-protocol population (PP) analysis may have fulfilled multiple reasons. MPM = malleable protein matrix (treatment); ITT = intention-to-treat cohort; FAS = full-analysis set.

the study with no major protocol deviations and were included in the PP population (63 subjects in the MPM and 68 in the placebo group).

Subject characteristics are provided in Table 2. About 51% of the ITT population was male. Average age was 54.5 ± 9.8 yr (range 27 to 81) and BMI 26.3 ± 3.6 kg/m². All subjects were of white ethnicity. Definition of race was by patient self-identification. Both groups were well balanced in terms of demographic and baseline data. Mean baseline LDL-C levels were 185 ± 32 and 173 ± 26 mg/dL in the MPM and placebo groups, respectively. Concomitant medications were equally distributed among the groups (not shown). Overall adherence was $91.7 \pm 17.2\%$ (median 98.2%) and $90.2 \pm 17.6\%$ (median 96.6%) in the MPM and placebo groups, respectively. Good adherence was seen in 87 and 81.5%, moderate adherence in 3.9 and 7.8%, and poor adherence in 9.1 and 10.4% of the MPM and placebo groups, respectively.

Diet Protocols

Results of the 3-d food records are given in Table 3. No significant differences in percentage changes from baseline were found for any macronutrients between the 2 treatment groups. However, total energy intake was significantly decreased by around 8% between baseline and visit 6 in the placebo group. Cholesterol intake (+11%) was significantly increased in the MPM group at the end of the treatment period compared with baseline. Both groups were below the daily cholesterol intake of 300 mg usually recommended (Lichtenstein et al., 2006).

Efficacy Analyses in the ITT Cohort

The average treatment duration was 78 ± 20 and 80 ± 18 d in the MPM and placebo groups, respectively. The percentage changes from baseline to study endpoint

Table 2. Demographic characteristics and baseline values of the intention-to-treat population¹

Item ²	Malleable protein matrix	Placebo	Total
n	77	77	154
Sex, n (male/female)	39/38	39/38	78/76
Age, yr	56 ± 9	53 ± 10	55 ± 10
Serum lipids, mg/dL			
TC	272 ± 35	260 ± 29	266 ± 33
LDL-C	185 ± 31	173 ± 26	179 ± 29
HDL-C	61 ± 14	61 ± 13	61 ± 14
TG	136 ± 65	127 ± 51	131 ± 59
ApoB	129 ± 26	120 ± 17	124 ± 22
TC/HDL-C	4.7 ± 1.2	4.4 ± 0.9	4.5 ± 1.1
LDL-C/HDL-C	3.2 ± 0.9	3.0 ± 0.8	3.1 ± 0.9
Glucose metabolism			
GLU, mg/dL	91 ± 8	91 ± 9	91 ± 8
INS, μ U/mL	5.6 ± 5.4	4.3 ± 2.7	5.0 ± 4.4
HOMA, μ U/mL $\times$ mg/dL	1.3 ± 0.9	1.0 ± 0.9	1.1 ± 1.0
HbA _{1c} , %	5.5 ± 0.4	5.5 ± 0.3	5.5 ± 0.3
Anthropometric data			
Body mass index, kg/m ²	26.3 ± 3.4	26.3 ± 3.9	26.3 ± 3.6
Waist circumference, cm	92.5 ± 11.4	93.3 ± 12.6	92.9 ± 12.0
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Other			
Systolic blood pressure, mmHg	129 ± 15	139 ± 19	130 ± 16
Diastolic blood pressure, mmHg	81 ± 9	81 ± 11	82 ± 9
C-reactive protein, mg/dL	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2

¹Data are means $\pm$ SD. To convert the values for cholesterol to mmol/L, multiply by 0.02586. To convert the values for TG to mmol/L, multiply by 0.01129.

²TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; ApoB = apolipoprotein B; GLU = fasting glucose; INS = fasting insulin; HOMA index = homeostatic model assessment (GLU $\times$ INS); HbA_{1c} = hemoglobin A_{1c}.

(last visit) and after 12 wk compared with placebo for lipid efficacy variables are shown in detail in Table 4. The relative TG reduction from baseline reached about 10% for MPM compared with placebo ($P = 0.019$). In the ITT cohort, TG decreased by $2.1\% \pm 28.4$ (-7.1 mg/dL) in the MPM arm and increased by $7.8\% \pm 29.5$ ($+9.3$ mg/dL) in the placebo arm. The primary out-

come, percentage change in LDL-C at study endpoint, did not reach statistical significance in the total cohort ($P = 0.781$), although distinct results were obtained in the university hospital outpatient clinic ($n = 76$, 49.3% of the ITT cohort), where a significant LDL-C decrease against placebo averaging between 5 and 10% after 3 wk ($P = 0.084$), 6 wk ($P = 0.016$), and 12 wk ($P =$

Table 3. Results of the 3-d food records at the end of the run-in period (before visit 3) and the end of the treatment period (before visit 6)¹

Nutrient intake, ² per d	MPM		Placebo		Treatment difference	
	Baseline	12 wk	Baseline	12 wk	Mean % (SD)	P-value
Total energy, kcal	1,867 (484)	1,786 (545)	2,009 (490)	1,801 ³ (511)	7.2 (24)	0.12
Total fat, % of energy	34.0 (5.1)	34.6 (6.8)	35.4 (7.5)	35.4 (7.2)	1.3 (26)	0.80
MUFA, % of fat	38.2 (4.4)	38.5 (4.3)	38.8 (5.4)	38.1 (4.3)	2.3 (15)	0.42
SFA, % of fat	42.1 (7.0)	42.4 (7.0)	41.5 (7.1)	42.0 (7.5)	1.1 (20)	0.78
PUFA, % of fat	19.6 (6.9)	19.1 (7.3)	19.5 (7.0)	19.9 (7.5)	-10.6 (42)	0.19
Carbohydrate, % of energy	46.1 (5.8)	45.3 (6.6)	44.5 (8.0)	44.7 (8.8)	-0.2 (16)	0.94
Protein, % of energy	16.4 (3.2)	17.3 ³ (3.4)	16.5 (3.4)	17.1 (4.4)	0.3 (27)	0.96
Alcohol, % of energy	3.5 (4.6)	2.7 ⁴ (3.5)	3.6 (4.7)	2.9 ^{3,4} (4.1)	0.13 (6)	0.37
Fiber, g	22.8 (8.4)	20.4 (7.9)	25.5 (28.3)	19.9 ³ (7.8)	4.0 (35)	0.55
Cholesterol, mg	256 (112)	285 ³ (139)	298 (130)	280 (169)	15.3 (69)	0.25

¹All values are means (SD).

²MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids. Total fat, carbohydrates, protein, and alcohol are given as percentage of total energy of the diet (kcal). The reported amounts are exclusive of the trial products.

³Significant change (%) from baseline at $P < 0.05$.

⁴Only includes subjects having reported some alcohol consumption at baseline.

0.058) was observed. No significant changes were found in TC, HDL-C, and ApoB concentrations, and the ratios of TC or LDL-C to HDL-C were not significantly altered.

For glucose metabolism variables (not shown in Table 4), GLU decreased slightly more in the MPM group than in the placebo group, although this difference was not statistically significant, whereas a small but significant difference in HbA_{1c} was observed after 12 wk of treatment ($P = 0.015$). The absolute percentage of HbA_{1c} was maintained at 5.5% in the MPM group but increased by 0.1 percentage unit from 5.5 to 5.6% in the placebo group. No significant relative changes were observed in INS or HOMA index. No significant changes were observed in BW, waist circumference, or waist-to-hip ratio. Furthermore, treatment did not affect BP or CRP.

Efficacy Analyses in Subjects with High TG at Baseline

Because most of the study participants in the ITT cohort were normo-triglyceridemic at baseline (TG <150 mg/dL, see Table 2), the treatment effect on lipid outcomes was further investigated in the subset of subjects having baseline TG levels of ≥ 150 mg/dL ($n = 42$, 27.3% of the ITT cohort). Demographic and baseline characteristics of this subset are shown in Table 5. About half of these subjects were meeting the metabolic syndrome criteria.

Percentage changes from baseline to study endpoint (last visit) and after 12 wk for lipid efficacy variables in this subset are shown in detail in Table 6, and the time course of relative TG decrease during the study comparing the ITT cohort and the high TG subset is shown in Figure 3. The treatment effect on TG reduction was much higher for subjects having TG levels at baseline of ≥ 150 mg/dL, reaching 20.0% compared with the placebo group ($P = 0.040$) after 12 wk. Concentrations of TG decreased from baseline by $12.7\% \pm 32.2$ (-32.6 mg/dL) in the MPM arm and increased by $7.3\% \pm 21.9$ ($+13.6$ mg/dL) in the placebo arm. Moreover, the maximum efficacy in TG decrease in the MPM group was apparently not reached after the study period, whereas a constant TG decrease was obtained after 6 and 12 wk following an initial increase after 3 wk of treatment (Figure 3). In this subset, the TG decrease was accompanied by a clear improvement in almost all lipid parameters, leading to decreases in TC (-7.5%), LDL-C (-5.5%), and especially TC – HDL-C (nHDL-C; -8.1%), despite the small decrease in HDL-C (-2.1%); these changes were all close to significance compared with placebo.

Table 4. Percentage changes from baseline to study endpoint and after 12 wk for treatment with malleable protein matrix (MPM) compared with placebo for lipid efficacy variables (intention-to-treat population)¹

Variable ²	MPM				Placebo				Treatment difference			
	Baseline		Treatment value		% change		Baseline		Treatment value		% change	
	Mean (SD)	Time point	Mean (SD)	n	Mean (SD)	n	Mean (SD)	Time point	Mean (SD)	n	Mean (SD)	P-value
LDL-C	185 (31)	EOT ³ 12 wk	179 (33) 176 (29)	77 69	-2.8 (12) -3.0 (13)	77	173 (26)	EOT 12 wk	169 (28) 170 (28)	77	-1.7 (12) -1.5 (12)	0.79 0.90
TC	272 (35)	EOT 12 wk	265 (42) 262 (38)	77 68	-2.2 (11) -2.5 (11)	77	260 (29)	EOT 12 wk	256 (33) 256 (35)	77	-1.4 (9) -1.2 (10)	0.90 0.73
TG	136 (65)	EOT 12 wk	130 (70) 127 (65)	77 68	-1.7 (28) -2.1 (28)	77	127 (51)	EOT 12 wk	135 (63) 134 (59)	77	6.7 (29) 7.8 (30)	0.03 0.012
HDL-C	61 (14)	EOT 12 wk	60 (18) 61 (19)	77 68	-1.4 (12) -1.2 (13)	77	61 (13)	EOT 12 wk	59 (13) 59 (13)	77	-2.8 (11) -3.0 (11)	0.57 0.69
TC/ HDL-C	4.7 (1.2)	EOT 12 wk	4.7 (1.2) 4.6 (1.1)	77 68	-0.2 (11) -0.7 (11)	77	4.4 (0.9)	EOT 12 wk	4.5 (1.0) 4.5 (1.0)	77	-2.1 (10) 2.4 (10)	0.43 0.39
ApoB	1.3 (0.3)	EOT 12 wk	1.2 (0.2) 1.2 (0.2)	75 68	-3.8 (10) -4.2 (10)	73	1.2 (0.2)	EOT 12 wk	1.2 (0.2) 1.1 (0.2)	73	-3.6 (11) -3.5 (11)	0.41 0.38

¹Baseline and treatment values are given in milligrams per deciliter.

²LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; ApoB = apolipoprotein B.

³EOT = end of treatment (treatment value at study endpoint).

Table 5. Demographic characteristics and baseline values of subjects having triglycerides (TG) ≥ 150 mg/dL at baseline¹

Item ²	TG ≥ 150 mg/dL		
	Malleable protein matrix	Placebo	ITT total
n/ITT (% ITT)	22/77 (28.6)	20/77 (26.0)	154
Sex, n (male/female)	10/12	14/6	78/76
Age, yr	53 $\pm$ 8	55 $\pm$ 11	55 $\pm$ 10
Serum lipids, mg/dL			
TG	220 $\pm$ 59	195 $\pm$ 47	131 $\pm$ 59
TC	294 $\pm$ 36	270 $\pm$ 31	266 $\pm$ 33
LDL-C	197 $\pm$ 31	177 $\pm$ 26	179 $\pm$ 29
HDL-C	55 $\pm$ 13	55 $\pm$ 11	61 $\pm$ 14
nHDL-C	239 $\pm$ 35	216 $\pm$ 31	205 $\pm$ 34
ApoB	147 $\pm$ 32	129 $\pm$ 17	124 $\pm$ 22
TC/HDL-C	5.5 $\pm$ 1.2	5.1 $\pm$ 0.9	4.5 $\pm$ 1.1
LDL-C/HDL-C	3.7 $\pm$ 1.0	3.4 $\pm$ 0.8	3.1 $\pm$ 0.9
Glucose metabolism			
GLU, mg/dL	91 $\pm$ 8	95 $\pm$ 7	91 $\pm$ 8
INS, μ U/mL	6.3 $\pm$ 3.8	5.0 $\pm$ 2.5	5.0 $\pm$ 4.4
HOMA, μ U/mL $\times$ mg/dL	1.4 $\pm$ 3.8	1.2 $\pm$ 0.6	1.1 $\pm$ 1.0
HbA _{1c} , %	5.6 $\pm$ 0.3	5.6 $\pm$ 0.3	5.5 $\pm$ 0.3
Anthropometric data			
Body mass index, kg/m ²	26.9 $\pm$ 3.1	27.8 $\pm$ 3.5	26.3 $\pm$ 3.6
Waist circumference, cm	95.2 $\pm$ 11.2	101.3 $\pm$ 10.5	92.9 $\pm$ 12.0
Other			
Systolic blood pressure, mmHg	132 $\pm$ 17	141 $\pm$ 20	130 $\pm$ 16
Diastolic blood pressure, mmHg	84 $\pm$ 10	86 $\pm$ 11	82 $\pm$ 9
CRP, mg/dL	0.3 $\pm$ 0.3	0.3 $\pm$ 0.1	0.3 $\pm$ 0.2
Metabolic syndrome ³	9/22 (40.9)	11/20 (55.0)	29/154 (18.8)
Individual criteria			
TG	22/22	20/20	20/29
Waist circumference	11/22	12/20	29/29
HDL	4/22	1/20	6/29
GLU	2/22	4/20	13/19
Blood pressure	17/22	17/20	28/29

¹Data are means $\pm$ SD.²ITT = intention-to-treat; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; nHDL-C = TC – HDL-C; ApoB = apolipoprotein B; GLU = fasting glucose; INS = fasting insulin; HOMA index = homeostatic model assessment (GLU $\times$ INS); HbA_{1c} = hemoglobin A_{1c}.³Number of subjects (% in parentheses) meeting at least 3 out of the 5 criteria for metabolic syndrome based on the set provided in the 2005 American Heart Association/National Heart, Lung, and Blood Institute statement (Grundy et al., 2005).

Safety and Adverse Events

A summary of the AE recorded during the study is given in Table 7. Overall, a total of 107 subjects (66.4%) experienced 1 serious adverse event (SAE) and 223 nonserious adverse events (AE). Fifty-three subjects (65.4%) in the MPM group and 54 subjects (67.5%) in the placebo group had AE or SAE. No treatment differences were found between the study groups regarding the number of subjects with at least one AE or SAE (Fisher's exact test: $P = 0.58$). The body system most frequently affected was the gastrointestinal system. No relevant differences were found between the 2 treatment groups regarding the frequency of most AE. No striking mean trends of clinical relevance were observed for laboratory variables or in any of the vital

signs during the course of the clinical trial, in either the MPM or the placebo group. Although tolerability was assessed as either good or excellent by 62 subjects in both treatment groups (80.5% of ITT cohort), it should be mentioned that the common gastrointestinal side effects, albeit mild and not posing serious safety concerns, may affect compliance in the real world.

DISCUSSION

Hypertriglyceridemia is implicated in the pathogenesis of CHD (Gouni-Berthold and Krone, 2005; Gandotra and Miller, 2008). Elevated TG at ≥ 150 mg/dL is closely linked to other atherogenic lipoproteins, such as very low density lipoproteins and remnants, and represents one of the major lipid biomarkers of

Table 6. Percentage changes from baseline to study endpoint and after 12 wk for treatment with malleable protein matrix (MPM) compared with placebo for all lipid efficacy variables of subjects having triglycerides ≥ 150 mg/dL at baseline

Item ¹	Time point	MPM		Placebo		Treatment difference	
		n	Mean % change (SD)	n	Mean % change (SD)	Mean % (SD)	P-value
TG	EOT ²	22	-9.7 (32)	20	8.5 (23)	-18.2 (28)	0.04
	12 wk	18	-12.7 (32)	17	7.3 (22)	-20.0 (28)	0.04
nHDL-C	EOT	22	-5.9 (14)	20	0.4 (14)	-6.3 (14)	0.14
	12 wk	18	-8.1 (14)	17	-0.0 (14)	-8.1 (14)	0.10
LDL-C	EOT	22	-5.2 (14)	20	-1.2 (14)	-4.0 (14)	0.36
	12 wk	18	-7.1 (14)	17	-1.5 (14)	-5.5 (14)	0.26
HDL-C	EOT	22	-2.5 (14)	20	-1.3 (14)	-1.2 (14)	0.78
	12 wk	18	-3.3 (15)	17	-1.2 (15)	-2.1 (15)	0.68
TC	EOT	22	-5.4 (13)	20	0.2 (13)	-5.7 (13)	0.15
	12 wk	18	-7.3 (13)	17	0.2 (13)	-7.5 (13)	0.10
TC/HDL-C	EOT	22	-5.4 (13)	20	0.2 (13)	-5.7 (13)	0.15
	12 wk	18	-3.3 (9)	17	-1.0 (9)	-2.2 (9)	0.45

¹TG = triglycerides; nHDL-C = TC - HDL-C; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol.

²EOT = end of treatment (treatment value at study endpoint).

the metabolic syndrome that altogether increase the risk of CHD. When combined with nonoptimal LDL-C level, TG ≥ 200 mg/dL was clearly defined as a lifestyle therapeutic target in the most recent NCEP ATP III report. As such, little evidence exists that the usual therapeutic dietary options for LDL lowering, such as plant stanols and sterols, dietary fiber, and soy proteins exert any beneficial effects on elevated TG. However, n-3 and fish oil supplements have shown modest TG decreases averaging -7% compared with those of placebos, especially in diabetic subjects with dyslipidemia,

but may concomitantly significantly increase LDL-C concentrations (Hartweg et al., 2009).

In the present controlled trial, MPM effectively decreased TG in subjects with hypercholesterolemia significantly beyond the effect of placebo. In the ITT cohort, the 12-wk treatment of moderately hypercholesterolemic subjects with 15 g of MPM twice daily significantly decreased TG by around 10% compared with placebo. This decrease reached 20% in subjects with TG ≥ 150 mg/dL at baseline. The primary outcome, defined as the relative change in LDL-C from baseline

Figure 3. Percentage change in triglycerides (TG) at each study visit (3, 6, and 12 wk) for the intention-to-treat (ITT) population and subjects having TG at baseline ≥ 150 mg/dL. MPM = malleable protein matrix (treatment).

Table 7. Incidence of adverse events (AE) following for treatment with malleable protein matrix (MPM) or placebo for 12 wk

Item	MPM		Placebo	
	AE, n	Subjects, n (%)	AE, n	Subjects, n (%)
All AE	109	53 (65.4)	113	54 (67.5)
Nonserious AE	109	53 (65.4)	112	53 (66.2)
Treatment-related AE ¹	41	31 (38.3)	37	24 (30.0)
Serious AE (SAE)	0	0 (0.0)	1	1 (1.2)
Treatment-related SAE ¹	0	0 (0.0)	0	0 (0.0)
Specific nonserious AE				
Gastrointestinal system	42	29 (35.8)	35	22 (27.5)
Infections and infestations	24	21 (25.9)	40	28 (35.0)
General disorders/ administration site conditions	12	10 (12.3)	14	13 (16.2)
Musculoskeletal and connective tissue disorders	6	5 (6.2)	4	4 (5.0)
Nervous system disorders	6	5 (6.2)	2	2 (2.5)

¹Causality to the study products rated as possible, probable, or certain.

to study endpoint, was not significantly changed in the present ITT cohort. A modest (averaging 5 to 10%) but significant decrease in LDL-C did occur over of the 12-wk study in subjects treated in one university lipid outpatient clinic that enrolled about 50% of the total study participants.

Supplementation of MPM has previously shown TG- and LDL-C-lowering properties as well as GLU and BP modulation in specific animal models (Beaulieu et al., 2010). Moreover, it was also shown that MPM, upon oral gavage in diet-induced obese mice, may act as a potential peroxisome proliferator-activated receptor gamma (PPAR γ) agonist (Lapointe et al., 2009). This could occur indirectly via the action of MPM on insulin or via reduction of specific cytokines and inflammation markers, considering the antiinflammatory effect of MPM and its ability to inhibit cytokine and chemokine production (i.e., IL-1 β , IL-6, and tumor necrosis factor- α) in murine immune disease models (Beaulieu et al., 2007b, 2009a).

The lipid-lowering effects of MPM might be attributable to a specific component of the product (whey proteins and peptides, bacteria cell debris, exopolysaccharides, vitamins, or minerals such as calcium), but most probably arise from the synergy among many of these components within MPM (Jacobs et al., 2009).

Several studies in animal models have investigated the lipid-lowering ability of whey proteins or specific peptides (as the main source of protein in the diet) with equivocal results (Sautier et al., 1983; Choi et al., 1989; Lapre et al., 1989; Nagaoka et al., 1991, 1992; Zhang and Beynen, 1993; Nagaoka, 1996; Kawase et al., 2000; Nagaoka et al., 2001; Pichon et al., 2008). Pal et al. (2010) showed that whey proteins significantly lower TC and LDL-C after a 12-wk treatment in overweight and obese individuals compared with placebo.

Pertaining to the possible role of high calcium intake in reducing serum lipids (Denke et al., 1993; Major et al., 2008), the isocaloric placebo used in this study providing 2,500 mg of calcium/d failed to show these benefits. A close and unique interaction of calcium with other components of the food matrix and especially whey proteins (Sun and Zemel, 2004; Pilvi et al., 2007), as found in MPM, may be required to observe a significant effect on serum cholesterol reduction and lipoproteins in humans. Also, many studies involving subjects with moderate hypercholesterolemia report lipid-lowering activities of fermented milk products containing live bacteria such as lactobacilli and bifidobacteria (Agerbaek et al., 1995; Richelsen et al., 1996; Schaafsma et al., 1998; Taylor and Williams, 1998; Gomes and Malcata, 1999; Agerholm-Larsen et al., 2000a,b; Sanders, 2000; St-Onge et al., 2000; Pereira and Gibson, 2002; Xiao et al., 2003), but it is unlikely that the observed effect of MPM on lipids come from such probiotic properties because the lactobacillus strain used in the production of MPM is heat-killed.

The primary outcome, defined as the relative change in LDL-C from baseline to study endpoint, was not significantly changed. Although MPM has been shown to reduce LDL-C by 32% in hamsters fed a high-fat, high-cholesterol diet (Beaulieu et al., 2010), the present study failed to reproduce a significant decrease in LDL-C in humans. However, a modest but highly significant decrease in LDL-C concentrations (averaging 5 to 7%) occurred in the subjects treated in one center (university lipid outpatient clinic), which enrolled 47% of the total subjects. Although inconsistencies in compliance assessment were documented in the nonuniversity centers, other reasons such as unrecognized recruiting heterogeneity or systematic differences in treatment circumstances across centers or type of medical set-

ting may explain these discrepancies (Localio et al., 2001). Therefore, further studies are needed to confirm whether MPM might have significant LDL-C-lowering effects in subjects with primary hypercholesterolemia.

Even though the observed decrease in LDL-C was small, it should be noted that even a 1 mg/dL decrease of LDL-C reduces the risk for developing CVD by about 1% (Grundy et al., 2004), thus making the potential LDL-C-lowering effects of MPM clinically relevant.

Furthermore, in the present study, no significant differences with regard to GLU and BP were detected, although GLU decreased slightly more in the MPM group than in the placebo group. The study participants were not selected at study entry based on these criteria and therefore most had normal glucose metabolism and were either normotensive or were already receiving antihypertensive therapy at baseline. Besides GLU and INS, regular consumption of MPM was associated with a small but significant (-0.1%) decrease in HbA_{1c}. Although this decrease is numerically small, even very small shifts of HbA_{1c} levels (0.1 to 0.2%) in the general population can dramatically affect the future incidence of CHD (Khaw et al., 2004). Finally, no treatment effect on anthropometric data (BMI, waist circumference, and waist-to-hip ratio) were shown; some evidence linking anorexigenic properties of whey protein-based products exists (Diepvens et al., 2008), but is still conflicting (Bowen et al., 2006a,b, 2007). However, the present study was not designed to measure short-term satiety and satiation or postprandial anorexigenic hormones, and no daily control on the food intake of the study participants was implemented.

Strengths and Limitations

The design used in the present study is the one generally accepted for studies investigating lipid-lowering agents (Berthold et al., 2006), and the trial was multi-center. Lipid and lipoprotein measurements were obtained on 2 different days at baseline (pooled baseline) to obtain reliable values before treatment. The measurements were done in a central, certified laboratory. The trial consisted of a placebo and diet run-in phase initiated 6 wk before baseline values were drawn; instructions to adhere to diet and lifestyle recommendations were given at the beginning of the 6-wk run-in phase. Diet had no influence on the results, as confirmed by comparison of the 3-d food diaries at the end of the run-in and treatment phases. The treatment duration was 12 wk; it might be argued that other effects such as those on BW, GLU, INS, or BP levels would take longer to develop.

CONCLUSIONS

Malleable protein matrix decreased TG in subjects with hypercholesterolemia significantly beyond the effects of placebo, especially in subjects having TG ≥ 150 mg/dL. Based on the LDL-C decrease obtained in the university lipid outpatient clinic in 50% of the study participants, further studies would be required to confirm whether MPM might have significant LDL-C-lowering effects in free-living primary hypercholesterolemic subjects. Malleable protein matrix could offer an effective TG-lowering dietary option for subjects not willing to participate in, or intolerant to, medical drug therapy. The TG-lowering properties of MPM should be further assessed in hypertriglyceridemic subjects such as those meeting criteria for metabolic syndrome or diagnosed with type 2 diabetes.

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