Wheat Germ Policosanol Failed to Lower Plasma Cholesterol in Subjects With Normal to Mildly Elevated Cholesterol Concentrations

Yuguang Lin, Mike Rudrum, Reggy P.J. van der Wielen, Elke A.Trautwein, Gerald McNeill, Aafje Sierksma, and Gert W. Meijer

Sugar cane policosanol, a mixture of long-chain primary alcohols (~67% as octacosanol), has been reported to lower plasma low-density lipoprotein (LDL)-cholesterol. We investigated the effect of wheat germ policosanol (WGP) on plasma lipid profiles in 58 adults (30 men and 28 women, aged 49 ± 11 years) with normal to mildly elevated plasma cholesterol concentrations in a double-blind, randomized, parallel placebo-controlled study. Subjects consumed chocolate pellets with or without 20 mg/d WGP for 4 weeks. Plasma lipid concentrations, routine blood chemistry and hematology were determined at the start and the end of the study. The initial plasma total, LDL-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triacylglycerol concentrations in the WGP and the control groups were identical. Over the 4 weeks, neither the WGP nor the control treatment significantly changed plasma total cholesterol, LDL- and HDL-cholesterol, or triacylglycerol concentrations when compared to baseline values. In addition, there was no significant difference in plasma lipid profiles between the WGP and the control groups at the end of the study. WGP did not result in any adverse effects as indicated by plasma activities of L-γ-glutamyltransferase (γ-GT), ALT, AST, bilirubin concentrations, and blood cell profiles. Chemical analysis showed that WGP consists of 8% hexacosanol, 67% octacosanol, 12% triacosanol, and 13% other long-chain alcohols, which is similar to the composition of sugar cane policosanol. In conclusion, WGP at 20 mg/d had no beneficial effects on blood lipid profiles. It therefore seems unlikely that the long chain (C24-34) alcohols have any cholesterol-lowering activity.

POLICOSANOL is a mixture of long chain (C24 to C34) primary alcohols, originally isolated from sugar cane wax.[1-3] These long-chain primary alcohols are also found in a number of other natural substances, such as bee wax, rice bran, and wheat germ.[4-6] Following the established trend[6] that the mixtures of long-chain alcohols are named "policosanols," herein we use a prefix to denote its natural origin, eg, sugar cane policosanol and wheat germ policosanol (WGP).

A number of studies showed that oral administration of sugar cane policosanol reduced plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations and increased high-density liporpotein (HDL)-cholesterol concentrations in healthy subjects,[7] hypercholesterolemics,[8] and type 2 diabetics,[9] as well as in a number of animal models.[2,10,11] This beneficial effect on plasma cholesterol was dose-dependent within the range 2 to 40 mg/d.[3] Reports indicate that the efficacy of sugar cane policosanol in improving the plasma lipid profile is equal to or even better than that of statins (such as lovastatin simvastatin, and pravastatin).[3,12,13] The underlying mechanisms by which sugar cane policosanol may lower plasma cholesterol are still unclear. A proposed mechanism is that policosanol may inhibit cholesterol biosynthesis as indicated by studies in cultured human fibroblasts[14] and in rabbits.[11] However, unlike statins, sugar cane policosanol is supposed to downregulate the cellular expression of hydroxymethylglutamyl coenzyme A (HMG-CoA) reductase rather than inhibit the enzyme activity.[15]

Since the chemical composition of WGP is similar to that of sugar cane policosanol, it may have similar beneficial effects as sugar cane policosanol. To our knowledge, no studies have investigated the potential cholesterol-lowering effect of policosanol derived from plant sources other than sugar cane in humans. In addition, all published studies describing the beneficial actions of sugar cane policosanol come from one research group, and have not yet been confirmed by other research laboratories.[3,16] Therefore, we conducted a randomized, double-blind, parallel placebo-controlled trial in adults with normal to mildly elevated plasma cholesterol concentrations to study the effects of WGP on plasma lipid concentrations. The results will provide further information on whether the long-chain primary alcohols are the bioactive compounds responsible for the reported beneficial effects of sugar cane policosanol.

MATERIALS AND METHODS

Subjects

This study was conducted in the Unilever Health Institute (previously Unilever Nutrition Center), Vlaardingen, The Netherlands. The ethical aspects of the study were approved by the Medical-Ethical Committee of Unilever Nederland BV. Volunteers were recruited by advertisements in local newspapers. Among the 84 volunteers who expressed their interest in the experiment, 60 eligible (based on following described criteria) subjects (30 men and 30 women) were selected. Study procedures were reviewed by these subjects and each provided written informed consent before protocol-specific procedures were performed. Participants were free to withdraw at any time during the study. The participants were between 18 and 70 years of age and had a body mass index between 19 and 30 kg/m^2. The range of plasma total cholesterol levels of the volunteers at baseline was 3.6 to 7.8 mmol/L. The volunteers had normal plasma activities of L-γ-glutamyltransferase (γ-GT; EC 2.3.2.2), AST (EC 2.6.1.1), and ALT (EC 2.6.1.2) at baseline. White blood cell, red blood cell, and platelet

From Unilever Health Institute, Unilever R&D, Vlaardingen, The Netherlands; and Loders Croklaan, Glen Ellyn, IL.

Submitted November 5, 2003; accepted May 3, 2004.

Presented in part at 13th International Symposium on Atherosclerosis in Kyoto, Japan, Sept 28–Oct 2, 2003. An abstract of this presentation has been published in Atherosclerosis 4:277, 2003 (suppl).

Address reprint requests to Yuguang Lin, PhD, Unilever Health Institute, Unilever R&D, PO Box 114, 3130 AC Vlaardingen, The Netherlands.

0026-0495/04/5310-0010$30.00/0

doi:10.1016/j.metabol.2004.05.006

counts, hemoglobin concentration, and hematocrit were all within the normal range. All of the participants had normal dietary patterns (none reported slimming diets, medically prescribed diets, vegan or vegetarian diets), and were in apparent good health, as self-reported. In addition, participants had no reported current disease or history of metabolic disease, chronic gastrointestinal disorders, cardiovascular disease, or high blood pressure; no reported medical treatment, and no use of medicine except analgesics or contraceptives. Alcohol intake was less than 21 U/wk for women and less than 28 U/wk for men. Volunteers had less than 10 hours per week of intense exercise. Female volunteers were not lactating or pregnant.

The volunteers were requested to retain their normal lifestyle (eg, activity) and habitual dietary pattern. During the study volunteers weekly answered a structured questionnaire on important deviations in lifestyle, as well as on intercurrent illnesses and medicine use. Volunteers did not donate blood except for the study or simultaneously participate in another biomedical trial.

Test and Control Chocolate Pellets

The test WGP was obtained from Garuda International, Inc (Santa Cruz, CA). Chocolate pellets with or without WGP were specially prepared by Loders Croklaan (Wormerveer, The Netherlands). Both types of chocolate pellets were identical in appearance and odor. All chocolate pellets were stored refrigerated (5°C) during the entire study. The long-chain primary alcohol (range, C24-34) content of the chocolate pellets was determined using a combination of high-performance liquid chromatography (HPLC) and gas chromatography (GC) techniques as described in the laboratory analysis section.

Study Procedures

This study was a double-blind, randomized, parallel placebo-controlled trial, which was performed according to good clinical practice and good laboratory practice guidelines. Sixty participants were stratified according to age, gender, and plasma lipid profiles and then randomly allocated to the two groups. Each participant received 4 chocolate pellets in a vial (labeled with blind codes) per day for 4 weeks. They were instructed to eat 2 chocolate pellets at breakfast and 2 at dinner and to fill in a compliance form. Body weight was measured at the start and the end of the study. Health status, medicine use, and deviations from the normal lifestyle were registered weekly by a questionnaire.

Laboratory Analysis

Assay of long-chain primary alcohols. Both octadecanol and behenyl alcohol were used as internal standards. After addition of the internal standards, each sample was extracted using 2 mL of 2:1 chloroform/methanol at 60°C for 5 minutes with frequent mixing using a Vibromix (Biolabo, Châtel-St-Denis, Switzerland). Then 8 mL of chloroform was added and the mixture extracted for another 10 minutes at 60°C. After lipid extraction, it was noted that the chocolate residues had not settled out so the solutions were filtered through fast filter paper. The solvent was removed under nitrogen. The recovered lipid extract was dissolved in 0.5 mL of chloroform. Aliquots, 25 μL, were injected onto a Varian 9010 HPLC system (Optimize Technologies, Oregon City, OR) and separated on a 125 mm $\times$ 4.0 mm Lichrosorb 5 SIL60A silica HPLC column (Chrompack, Raritan, NJ). The long-chain alcohols were collected from the HPLC column as a single fraction. After removal of the solvent, the recovered material was silylated with 400 μL BSTFA (N,O-bis(trimethylsilyl) trifluoracetamide) and 200 μL pyridine for 30 minutes at 80°C. The silylation reagents were removed under nitrogen and the residues were dissolved in 200 μL toluene. A 15-μL aliquot of this solution was injected onto the GC system. GC separations were performed using a Perkin Elmer 8500 series GC fitted with a 10 m $\times$ 0.53 mm id Quadrex DB5 widebore capillary column (Norwalk, CT). An oven temperature program of 120 to 250°C/10°C per minute, followed by 250 to 355°C/15°C per minute with a 3-minute hold at 355°C was used.

Blood sampling. On the night preceding the blood sampling, volunteers were instructed not to eat anything after 8 PM and not to drink anything after 10 PM, except water. Blood (6 mL) was sampled from the antecubital vein for biochemical and hematological assays. Plasma was prepared by centrifugation for 10 minutes at 3,000 rpm and was divided into 4 portions for different determinations. Plasma was stored at -20°C until analysis.

Plasma lipids. Total cholesterol concentration was determined by an enzymatic method by using a commercial total cholesterol testkit (CHOD-PAP; Boehringer, Mannheim, Germany). HDL-cholesterol was measured as described for total cholesterol after precipitation of very-low-density liporptein (VLDL)-cholesterol and LDL-cholesterol with phosphotungstate and magnesium.

Plasma LDL-cholesterol was calculated by the Friedewald equation.[17] Plasma total and free glycerol concentrations were analyzed according to the glycerol phosphate oxidase (GPO)-Trinder (PAP) method using a total glycerol testkit (Roche, Basel, Switzerland) and a test kit for free glycerol (Sigma, St Louis, MO). Plasma triacylglycerol concentration was calculated as plasma total glycerol (mmol/L) minus plasma free glycerol (mmol/L).

All laboratory analyses were performed on a Cobas Mira S automated analyser (Roche, Basel, Switzerland).

Enzymes. In plasma, activity of γ-GT was determined with the International Federation for Clinical Chemistry (IFCC) method using a commercial test kit (Boehringer). The plasma activities of AST and ALT were determined using commercial test kits (Roche).

Bilirubin. Plasma levels of bilirubin were directly determined according to the dichlorophenyl diazonium method using a total bilirubin testkit (Boehringer).

Hematology. Hemoglobin concentration, white blood cell, red blood cell, and platelet counts, and hematocrit were all determined on a Sysmex F-800 blood cell counter (Toa Medical Electronics, Kobe, Japan).

Dietary Intake Assessment

At the end of the study dietary intake was assessed by a modification of the Valivet method (Nutri-AKT, Rhenoy, The Netherlands). This method has been developed and validated by the Department of Human Nutrition of the Wageningen Agricultural University.[18] A food frequency questionaire with 104 items specifically aimed at estimating fat intake. We modified the method slightly by extending the number of margarines and oils to cover the range of products used by the participants and we substituted more recent analytical data of fat composition of a variety of products for older data in the food composition database. Subjects filled in the food frequency questionnaire. The answers were checked for completeness during an interview with a dietician.

Statistical Analysis

Calculation of sample size was based on an expected within-person variation of plasma cholesterol concentrations observed in a previous study (0.35 mmol/L).[19] Thirty volunteers per group would enable detection of a difference of 0.4 mmol/L total cholesterol with a power of 0.90 and $\alpha = 0.05$. Data of plasma lipid profiles and clinical blood parameters are presented as mean and pooled SE, which makes comparison of differences between 2 groups easier (the 95% confidence interval for a difference between 2 mean values is roughly ± 3 pooled SE). Other data are presented as means $\pm$ SD. The differences of mean values between the control and WGP groups were determined using Student's *t* test. The chi-square test was used to compare adverse event between the 2 groups. A *P* value less than .05 was considered statis-

Table 1. Characteristics (mean ± SD) of Study Volunteers

Characteristic	Control Group	WGP Group
Male/female	15/14	15/14
Age (yr)	49 ± 11	49 ± 11
Body weight (kg)	75.4 ± 10.4	76.5 ± 7.8
Body height (m)	1.75 ± 0.10	1.74 ± 0.07
Body mass index (kg/m^2)	24.5 ± 2.1	25.3 ± 2.0
Sporting activities (h/wk)	1 ± 1	1 ± 1
Alcoholic beverage consumption (U/wk)	7 ± 7	4 ± 5

tically significant. SAS version 6.12 was used for analyses (SAS Institute, Cary, NC).

RESULTS

Volunteer Characteristics and Compliance

The study started with 60 volunteers. Two female volunteers dropped out (one from each group) due to illnesses and associated medicine use not related to the treatment of this study. The characteristics (at the start of the trial) of the volunteers who completed the entire study are summarized in Table 1. The average body weight at start of the study was 75.4 kg in the control group and 76.5 kg in the WGP group, respectively.

None of the volunteers started a diet or changed their eating habits during the experiment according to the weekly questionnaire about lifestyle. During the study the same individuals smoked and the amount smoked per day did not change for each smoker (data not shown). No differences were found in the hours of exercise during the study within each person. In concert with these results, the body weight of the volunteers was not changed during the 4-week experiment. The final body weight was 75.6 kg in control and 76.2 kg in the WGP groups, respectively. Eleven subjects in the WGP group reported having mild illnesses such as headache/migraine, gastrointestinal complaints, and common cold during the trial period, compared to seven subjects in control group. However, differences of reported illnesses between the 2 groups were not statistically significant ($P < .20$).

Compliance with the treatment was excellent in both groups. According to the chocolate pellets compliance forms and counting the chocolate pellets left over, 0% of the fortified pellets and 0.4% of the control pellets were not eaten (data not shown). Reason for not eating was forgetfulness. More than 99.6% of the pellets were eaten at breakfast and dinner, as instructed in both groups.

Table 2. Comparison of Composition Between WGP and Sugar Cane Policosanol

Components	% of Total Long-Chain Alcohols	
	WGP	Sugar Cane Policosanol*
Hexacosanol (C26)	8.4	7-8
Octacosanol (C28)	67.9	66-67
Triacosanol (C30)	12.6	12-14
Other long-chain alcohols	11.1†	11-15

NOTE. The values of WGP are means of 2 independent determinations using a combination of HPLC and GC as described in the Methods.

*Reported data.[1,2]

†Including C24 (2.1%), C32 (5.9%), and C34 (3.1%).

Table 3. Composition of Control and WGP-Enriched Chocolate Pellets

Composition (g/100 g)	Control Pellets	WGP Pellets
Sugar	48	48
CLSP 555/E*	30.7	30.7
Cocoa powder	14	14
Skimmed milk powder	7	7
Vanilla	0.02	0.02
Lecithin	0.4	0.4
WGP	—	0.345

*Dry fractioned palm kernel stearin (99%, w/w %) + 1 % emulsifier.

Compositions of WGP and Chocolate Pellets

The composition of WGP is shown in Table 2. The results showed that the mixture of long-chain alcohols in WGP has a similar composition to that reported in the literature for sugar cane policosanol.[20] Also, the percentage long-chain alcohols is similar in WGP and sugar cane policosanol (95% *v* 97%).[2,3]

Table 3 shows the composition of the chocolate pellets. The content of WGP was based on the analytical data. Except for WGP, the compositions of the experimental and control chocolate pellets were identical. Based on the average weight of the test chocolate pellets (1.45 g) and its WGP concentration (0.345%), 20 mg WGP (carried in 4 pellets) was ingested per day by each volunteer in the WGP group.

Plasma Lipid Concentrations

Table 4 lists the blood lipid data at the beginning and at the end of the study. Over the 4 weeks, neither the WGP nor the

Table 4. Plasma Lipid Concentrations in Control and WGP Groups

	Control	WGP	Pooled SE
Total cholesterol (mmol/L)			
Week 0	5.58	5.56	0.189
Week 4	5.59	5.44	0.189
Change	0.01	−0.12	0.084
LDL-cholesterol (mmol/L)			
Week 0	3.65	3.63	0.172
Week 4	3.59	3.54	0.175
Change	−0.06	−0.09	0.067
HDL-cholesterol (mmol/L)			
Week 0	1.27	1.27	0.064
Week 4	1.30	1.25	0.057
Change	0.03	−0.02	0.026
LDL/HDL ratio			
Week 0	3.06	3.14	0.220
Week 4	2.96	3.04	0.214
Change	−0.10	−0.11	0.083
Triacylglycerol (mmol/L)			
Week 0	1.47	1.46	0.108
Week 4	1.57	1.44	0.127
Change	0.11	−0.02	0.069

NOTE. No statistically significant differences were found between control and WGP groups for all parameters.

Table 5. Clinical Chemical and Hematological Parameters in Control and WGP Groups

	Control Group	WGP Group	Pooled SE
γ-GT (μkat/L)			
Week 0	0.53	0.46	0.055
Week 4	0.48	0.45	0.044
Change	−0.04	−0.01	0.026
Alanine aminotransferase (μkat/L)			
Week 0	0.27	0.30	0.022
Week 4	0.32	0.30	0.021
Change	0.05	0.00	0.020
AST (μkat/L)			
Week 0	0.32	0.32	0.018
Week 4	0.26	0.24	0.020
Change	−0.06	−0.08	0.019
Bilirubin (total) (μmol/L)			
Week 0	13.1	14.0	13.1
Week 4	12.4	13.3	12.4
Change	−0.7	−0.7	−0.72
White blood cells (10^9/L)			
Week 0	7.22*	6.20*	0.275
Week 4	7.31*	6.02*	0.277
Change	0.09	−0.18	0.213
Red blood cells (10^{12}/L)			
Week 0	4.76	4.85	0.069
Week 4	4.74	4.85	0.070
Change	−0.03	0.00	0.035
Hemoglobin (mmol/L)			
Week 0	9.02	9.01	0.127
Week 4	9.01	9.07	0.134
Change	−0.01	0.06	0.052
Hematocrit			
Week 0	0.439	0.439	0.0055
Week 4	0.437	0.438	0.0062
Change	−0.001	0.00	0.0031
Platelets (10^9/L)			
Week 0	274	253	11.3
Week 4	257	252	10.1
Change	−17	−0.97	6.57

*Indicates statistically significant differences between WGP and control groups ($P < .05$).

control treatment significantly changed plasma total cholesterol, LDL- and HDL-cholesterol, or triacylglycerol concentrations when compared to baseline values. Therefore, there was no difference in plasma lipid profiles between the WGP group and the control group either at the beginning or at the end of the study.

Clinical Chemistry and Hematology

All values of the blood parameters, as assessed at the beginning and the end of the study, were similar and within the normal ranges (Table 5). There were no differences of these enzymic and hematological variables between the control and the WGP groups, except that control group has higher white blood cell counts ($P < .05$). Nevertheless, there was no significant difference in the changes in white blood cell counts between the groups in the 4-week trial.

Dietary Energy and Lipid Intake

The estimated dietary energy and lipid intake is presented in Table 6. Chocolate pellets contributed approximately 2% of total dietary energy. No statistically significant differences were found in energy intake, total fat intake (in percentage of energy intake), and fat composition (saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids) and dietary cholesterol intake between the 2 groups.

DISCUSSION

Although policosanol naturally exists in the wax fraction of plant foods, its concentration in food is low.[16] This study for the first time investigated the potential short-term adverse effects of concentrated wheat germs policosanol used as a supplement. Our results show that WGP caused apparently no adverse effects in the volunteers as indicated by plasma hepatic enzyme activities, blood cell profiles, and self-reported health status. For each volunteer, the activities of plasma ALT, AST, and γ-GT, and blood cell profiles were within the normal range at the beginning and the end of the trial. These data are in agreement with results of toxicity studies of sugar cane policosanol in animal models, including mice,[20] rats,[21] rabbits,[5] dogs,[6] and monkeys.[2] A long-term (>2 years) clinical study also showed that the incidence of adverse effects (none serious) was similar between sugar cane policosanol and control groups.[3] We do not have an explanation for the observed significant difference in white blood cell counts between the 2 groups, which persisted from the beginning to the end of the study. However, the higher white blood cell counts in control group indicates that this phenomenon is not relevant to WGP supplementation.

In the present double-blind, randomized, parallel placebo-controlled study, we found that dietary supplementation of 20 mg/d WGP for 4 weeks did not alter plasma total or LDL-cholesterol concentrations in adults with normal to mildly elevated plasma cholesterol concentrations. Plasma triacylglycerol concentration was also not changed by WGP. These results contrast with reported studies in animals and humans, where sugar cane policosanol lowered both plasma total and LDL-cholesterol and triacylglycerol concentrations.[3,22-26] However, our results are in agreement with a recent animal study which demonstrated that neither sugar cane policosanol nor rice wax policosanol lowered plasma total or LDL-cholesterol in hamsters.[6]

Octacosanol, which accounts for about 67% of sugar cane

Table 6. Dietary Energy and Lipid Intake (mean ± SD) During the Experiment in Control and WGP Groups

	Control Group	WGP Group
Energy (kJ/d)	9,369 ± 300	9,492 ± 404
Fat (en%)	40.8 ± 1.1	39.6 ± 1.1
Saturated fatty acids (en%)	16.4 ± 0.50	16.4 ± 0.56
Monounsaturated fatty acids (en%)	13.2 ± 0.38	12.9 ± 0.34
Polyunsaturated fatty acids (en%)	9.6 ± 0.43	8.9 ± 0.47
Cholesterol (mg/d)	221 ± 10	217 ± 8.3

NOTE. No statistically significant differences were found between control and WGP groups for all parameters.

Abbreviation: en%, energy %.

policosanol, has been suggested to be the active ingredient responsible for the cholesterol-lowering effect of sugar cane policosanol.[27] In this study, WGP contained 68% octacosanol and the other long-chain alcohol components were also similar to those reported for sugar cane policosanol. There is no obvious explanation for the different effects on lipid metabolism that have been found between WGP and sugar cane policosanol. Sugar cane policosanol apparently lowers plasma cholesterol in a dose-dependent manner (within 2 to 40 mg/d).[3] In the present study, an intake level of 20 mg/d WGP was tested. The lack of a cholesterol-lowering effect of WGP is therefore unlikely due to a too low dose. In addition, the cholesterol-lowering effect of sugar cane policosanol was reported independent of the initial plasma cholesterol concentrations.[3] In normocholesterolemic and hypercholesterolemic subjects, sugar cane policosanol has been reported to result in a similar cholesterol-lowering efficacy.[3] Therefore, the background of the volunteers' plasma cholesterol concentrations cannot account for the negative results in this study. Additionally, in the present study WGP did not lower cholesterol in subjects ($n = 21$) with elevated baseline total cholesterol ($\geq$5.2 mmol/L) as compared to those ($n = 8$) with normal baseline total cholesterol ($<$5.2 mmol/L) (data not shown).

In the reported studies sugar cane policosanol was usually administrated in the form of a tablet. In the present study chocolate pellets were used as carrier of WGP. It seems unlikely that the chocolate pellets could have counteracted the blood cholesterol-lowering effect of WGP, as we did not find changes in plasma cholesterol concentrations in the control group. In addition, the production procedure of chocolate pellets did not change the chemical structures of WGP as indicated by the results of the chromatographic assay (Table 2). It has been reported that policosanol is very stable, and remained unchanged even in extreme conditions as tested by acid and alkaline hydrolysis, oxidative and photolytic degradation, and thermal degradation.[28] As shown in Table 3, the chocolate pellets contained common food ingredients. It is unlikely that these ingredients would affect absorption of policosanol. To our knowledge no studies have reported blood policosanol concentration in subjects ingesting sugar cane policosanol. Studies in human and animals with oral administration of [^{3}H]-octacosanol showed that the majority (81% to 91%) of total radioactivity was excreted in the faeces and only 1.2% (in humans) to 3.3% (in rats) of total radioactivity was found in urine.[29] From these data it can be concluded that the absorption rate of policosanol is very low. Also, it should be taken into account that the absorbed radioactivity may not represent octacosanol. This radioactivity may come from products of [^{3}H]-octacosanol breakdown in the gut.

The volunteer compliance in this study was excellent. The chocolate bean consumption and habitual dietary intake was not statistically significantly different between the 2 groups. The average total energy intake was 9,369 and 9,492 kJ/d in the control and the WGP groups, respectively, and fat contributed 40% to 41% of total energy. Furthermore, body weights of the volunteers were constant during the study. These data support the volunteer's self-report that they kept their habitual lifestyles during the trial.

It has been reported that on a weight-by-weight comparison sugar cane policosanol lowers plasma cholesterol to the extent similar to, or even better than, that of statins (including lovastatin, simvastatin, atorvastatin, and pravastatin) in human studies.[3,12,13] However, unlike statins, which inhibit the activity of HMG-CoA reductase, the mechanism underlying the putative cholesterol-lowering effect of sugar cane policosanol has not yet been established. Menedeze et al[14,30] reported that policosanol inhibited cholesterol biosynthesis from acetate but not from mevalonate in cultured human fibroblasts, suggesting an inhibition of HMG-CoA reductase. A subsequent study showed that policosanol did not directly inhibit HMG-CoA reductase.[15] An explanation for that could be that policosanol might suppress the activity of HMG-CoA reductase by downregulating its cellular expression.[15] Hepatic cholesterol synthesis was reported to be suppressed by policosanol in diet-induced hypercholesterolemic rabbits.[11] Recently, Wang et al[6] demonstrated that neither sugar cane policosanol nor rice wax policosanol was able to reduce body cholesterol biosynthesis in hamsters. Thus it is not established with certainty that sugar cane policosanol inhibits cholesterol biosynthesis.

As reviewed by Janikula[3] and Yatcilla,[16] all published clinical studies about the cholesterol-lowering effect of sugar cane policosanol were conduced by one laboratory or sponsored by one company. To date no other laboratories have confirmed the beneficial effect of sugar cane policosanol in well-designed clinical studies. The present study demonstrated that WGP does not lower plasma cholesterol concentrations in human adults with normal to mildly elevated plasma cholesterol concentrations. As described here, the chemical compositions are similar between sugar cane policosanol and WGP. Our results challenge the reported beneficial effects of sugar cane policosanol.

In conclusion, WGP at a dose of 20 mg/d had no beneficial effects on blood lipid profiles in this human study. It therefore seems unlikely that octacosanol or other long-chain (C24-34) alcohols have any cholesterol-lowering activity.

ACKNOWLEDGMENT

The authors thank Tom Wiersma and Henk van der Knaap for their excellent assistance in statistical analysis.

REFERENCES

1. Arruzazabala ML, Noa M, Menendez R, et al: Protective effect of policosanol on atherosclerotic lesions in rabbits with exogenous hypercholesterolemia. Braz J Med Biol Res 33:835-840, 2000

2. Rodriguez-Echenique C, Mesa R, Mas R, et al: Effects of policosanol chronically administered in male monkeys (Macaca arctoides). Food Chem Toxicol 32:565-575, 1994

3. Janikula M: Policosanol: A new treatment for cardiovascular disease? Altern Med Rev 7:203-217, 2002

4. Aleman C, Rodeiro I, Noa M, et al: One-year dog toxicity study of D-002, a mixture of aliphatic alcohols. J Appl Toxicol 21:179-184, 2001

5. Rodriguez MD, Gamez R, Sanchez M, et al: Developmental toxicity of D-002 (a mixture of aliphatic primary alcohols) in rats and rabbits. J Appl Toxicol 18:313-316, 1998

6. Wang YW, Jones PJ, Pischel I, et al: Effects of policosanols and phytosterols on lipid levels and cholesterol biosynthesis in hamsters. Lipids 38:165-170, 2003

7. Arruzazabala ML, Molina V, Mas R, et al: Antiplatelet effects of policosanol (20 and 40 mg/day) in healthy volunteers and dyslipidaemic patients. Clin Exp Pharmacol Physiol 29:891-897, 2002

8. Pons P, Rodriguez M, Robaina C, et al: Effects of successive dose increases of policosanol on the lipid profile of patients with type II hypercholesterolaemia and tolerability to treatment. Int J Clin Pharmacol Res 14:27-33, 1994

9. Castano G, Fernandez L, Mas R, et al: Comparison of the efficacy, safety and tolerability of original policosanol versus other mixtures of higher aliphatic primary alcohols in patients with type II hypercholesterolemia. Int J Clin Pharmacol Res 22:55-66, 2002

10. Arruzazabala ML, Carbajal D, Mas R, et al: Cholesterol-lowering effects of policosanol in rabbits. Biol Res 27:205-208, 1994

11. Menendez R, Arruzazabala L, Mas R, et al: Cholesterol-lowering effect of policosanol on rabbits with hypercholesterolaemia induced by a wheat starch-casein diet. Br J Nutr 77:923-932, 1997

12. Ortensi G, Gladstein J, Valli H, et al: A comparative study of policosanol versus simvastatin in elderly patients with hypercholesterolemia. Curr Therapeut Res 58:390-401, 1997

13. Castano G, Mas R, Fernandez L, et al: Comparison of the efficacy and tolerability of policosanol with atorvastatin in elderly patients with type II hypercholesterolaemia. Drugs Aging 20:153-163, 2003

14. Menendez R, Fernandez SI, Del Rio A, et al: Policosanol inhibits cholesterol biosynthesis and enhances low density lipoprotein processing in cultured human fibroblasts. Biol Res 27:199-203, 1994

15. McCarty MF: Policosanol safely down-regulates HMG-CoA reductase—Potential as a component of the Esselstyn regimen. Med Hypoth 59:268-279, 2002

16. Yatcilla M: Policosanols, cholesterol solution or controversial problem? Nutraceuticals World April:28-34, 2002

17. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972

18. Feunekes GI, Van Staveren WA, de Vries JH, et al: Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 58:489-496, 1993

19. Weststrate JA, Meijer GW: Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 52:334-343, 1998

20. Aleman CL, Puig MN, Elias EC, et al: Carcinogenicity of policosanol in mice: an 18-month study. Food Chem Toxicol 33:573-578, 1995

21. Rodeiro I, Aleman C, Noa M, et al: Preclinical oral toxicology in rats of D-002, a natural drug with antiulcer effects. Drug Chem Toxicol 21:151-162, 1998

22. Gouni-Berthold I, Berthold HK: Policosanol: Clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am Heart J 143:356-365, 2002

23. Alcocer L, Fernandez L, Campos E, et al: A comparative study of policosanol versus acipimox in patients with type II hypercholesterolemia. Int J Tissue React 21:85-92, 1999

24. Batista J, Stusser R, Saez F, et al: Effect of policosanol on hyperlipidemia and coronary heart disease in middle-aged patients. A 14-month pilot study. Int J Clin Pharmacol Ther 34:134-137, 1996

25. Canetti M, Moreira M, Mas R, et al: A two-year study on the efficacy and tolerability of policosanol in patients with type II hyperlipoproteinaemia. Int J Clin Pharmacol Res 15:159-165, 1995

26. Castano G, Mas R, Arruzazabala ML, et al: Effects of policosanol and pravastatin on lipid profile, platelet aggregation and endothelemia in older hypercholesterolemic patients. Int J Clin Pharmacol Res 19:105-116, 1999

27. Arruzazabala MD, Carbajal D, Mas R, et al: Effects of D-003, a new compound purified from sugarcane wax, on platelet aggregation in healthy volunteers—A randomised, double-blind clinical study. Clin Drug Invest 23:107-118, 2003

28. Cabrera L, Rivero B, Magraner J, et al: Stability studies of tablets containing 5 mg of policosanol. Boll Chim Farm 142:277-284, 2003

29. Mas R: Policosanol. Drugs Future 25:569-586, 2000

30. Menendez R, Amor AM, Rodeiro I, et al: Policosanol modulates HMG-CoA reductase activity in cultured fibroblasts. Arch Med Res 32:8-12, 2001