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Policosanol Has No Antioxidant Activity in Human Low-Density Lipoprotein but Increases Excretion of Bile Acids in Hamsters

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Policosanol is a group of long chain primary alcohols and has been shown to reduce blood cholesterol levels and to inhibit the oxidation of low-density lipoprotein (LDL). The present study examined (i) the effect of policosanol supplementation in the diet on the fecal excretion of neutral and acidic sterols in hamsters and (ii) the antioxidant activity of policosanol in human LDL. Golden Syrian hamsters were divided into four groups (n = 12/each) fed one of the four diets containing 0 (control), 0.38, 0.75, and 1.50 g kg^{-1} policosanol for 6 weeks. It was found that hamsters given 0.38−1.5 g kg^{-1} diets had a serum total cholesterol level lowered by 15−25% and had a high-density lipoprotein cholesterol elevated by 7−16.8%. It was found that policosanol increased the excretion of acidic sterols by 25−73%. Contrary to that in previous reports, policosanol had no apparent anti-LDL oxidation activity when 1-tetracosanol, 1-hexacosanol, and 1-octacosanol were incubated in human LDL. Policosanol also possessed no scavenging activity on the free radical2,2-diphenyl-1-picrylhydrazyl. These data provide evidence that in addition to the effect of HMG-CoA reductase, the cholesterol-lowering activity of policosanol is partially mediated by its inhibition on the absorption of bile acids, but these data disprove the claim that policosanol is an antioxidant.

KEYWORDS: Cholesterol; HDL; LDL; octacosanol; policosanol; primary alcohol; triacontanol; triglyceride

INTRODUCTION

Cardiovascular disease is a leading cause of death in many developed countries. High blood cholesterol levels are the major risk in the contribution to cardiovascular disease. In recent years, many natural compounds have attracted much interest due to their potential as functional neutraceuticals to treat hypercholesterolemia. Policosanol refers to a mixture of long chain primary alcohols (C24−34), originally isolated from sugar cane, rice bran, beeswax, sorghum kernel, and wheat germ. Policosanol has been shown to lower plasma total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol and to elevate high-density lipoprotein cholesterol (HDL-C) in type 2 diabetics (*1*) and hypercholesterolemia patients (*2*, *3*). A favorable effect of policosanol on plasma lipids has also been demonstrated in rabbit (*4*), monkey (*5*), dog (*6*), and rat (*7*). However, the cholesterol-lowering effect associated with policosanol was not always observed in other studies (*8*, *9*).

The mechanism by which policosanol reduces plasma TC remains poorly understood. It has been shown that policosanol reduces cholesterol synthesis by down-regulation of 3-hydroxy-3-methylglutary coenzyme A (HMG-CoA) reductase (*10*, *11*). Policosanol has also been shown to increase the clearance of LDL-cholesterol (LDL-C) by increasing LDL binding, uptake, and degradation (*12*). In addition, policosanol has been demonstrated to inhibit LDL oxidation (*13*) and to decrease platelet aggregation and production of thromoboxane B_2 (*14*, *15*).

The blood cholesterol level can be lowered if either cholesterogenesis or absorption of cholesterol and bile acids is inhibited. The present study used hamsters as an animal model to study further the effect of policosanol on serum cholesterol level and fecal excretion of neutral and acidic sterols. It was the first time to demonstrate that the cholesterol-lowering activity of policosanol was also partially mediated by its inhibition on the absorption of acidic sterols. The present study reassessed the antioxidant activity of policosanol and found that it had no free radical scavenging activity and exhibited no effect on LDL oxidation.

MATERIALS AND METHODS

Chemicals. A sugar cane policosanol mixture was obtained from Lithy Fine Chemical Co., Ltd. (Shanghai, China). α-Tocopherol and three policosanols with 99% purity, namely, 1-tetracosanol, 1-hexacosanol, and 1-octacosanol, were purchased from Sigma (St. Louis, MO).

Diets. The formula previously described by Zhang et al. (*16*) was modified to prepare four diets for hamsters. The control diet was prepared by mixing all powdered ingredients and lard listed in **Table 1** except for gelatin. Three experimental diets were prepared similarly by adding 0.38 (P0.38), 0.75 (P0.75), and 1.5 g (P1.50) of policosanol into 1 kg of control diet, respectively. All four diets contained 0.1% cholesterol. All four powered diets were then mixed with a gelatin

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Table 1. Composition of the Control Diet and the Experimental Diets Supplemented with Policosanol in 1 kg Diet

	control	P0.38	P0.75	P1.50
cornstarch (g)	508	508	508	508
casein (g)	242	242	242	242
sucrose (g)	119	119	119	119
lard (g)	50	50	50	50
mineral mix (AIN-76) (g)	40	40	40	40
vitamin mix (AIN-76A) (g)	20	20	20	20
gelatin	20	20	20	20
DL-methionine (g)	1	1	1	1
cholesterol (g)	1	1	1	1
total policosanol (g)	0	0.38	0.75	1.50
octacosanol (C28)	0	0.20	0.39	0.78
triacontanol (C30)	0	0.16	0.32	0.64
others	0	0.02	0.04	0.08

solution (20 g L^{-1}) in a ratio of 200 g diet per liter of solution. Once the gelatin had set, the diets were cut into approximately 20 g cubic portions and stored frozen (−20 °C).

Animals. Forty-eight male Golden Syrian hamsters (*Mesocricetus auratus*, 106 ± 8 g; 10 weeks, Laboratory Animal Service Center, The Chinese University of Hong Kong) were divided into four groups (n = 12) fed one of the four diets. All hamsters were housed (two per cage) in an animal room at 23 °C with 12/12 h light−dark cycles. The fresh diets were given daily, and uneaten food was discarded. The food intake was measured daily, and the body weight was recorded twice a week. The hamsters were given free access to food and fluid. The total fecal output of each cage was combined. All of the hamsters were bled from the retro-orbital sinus into a heparinized capillary tube under light ether anesthesia after overnight fasting at weeks 0, 2, 4, and 6 (*17*). After it clotted, the blood was centrifuged at 1500*g* for 10 min and serum was collected. At the end of week 6, all of the hamsters were killed; liver, heart, kidney, and adipose tissues (epididymal and prerenal pads) were removed, washed in saline, weighed, and frozen in liquid nitrogen. All samples were stored at −80 °C in a freezer prior to cholesterol analysis.

Serum Lipids. Serum TC and triglyceride (TG) levels were determined enzymatically by using commercial kits (Sigma Chemical). HDL-C was measured after precipitation of LDL and very LDL with phosphotungstic acid and magnesium chloride (Sigma). Non-HDL-C was calculated by deducting HDL-C from the TC.

Determination of Cholesterol in Liver, Heart, and Adipose Tissues. The total lipids were extracted from 300 mg of tissue sample with the addition of 1 mg of stigmastanol as an internal standard, using 15 mL of chloroform−methanol (2:1, v/v). The lipid extracts were then saponified with 6 mL of 1 N NaOH in 90% ethanol at 90 °C for 1 h, and the nonsaponfied substances including cholesterol were then converted to their trimethyl-silyl (TMS)-ether derivatives by a commercial TMS reagent (Sigma). Analysis of the cholesterol TMS-ether derivative was performed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) in a Shimadzu GC-14B GLC equipped with a flame ionization detector (Shimadzu, Tokyo, Japan). The column temperature was set at 285 °C and maintained for 30 min. Helium was used as the carrier gas at a head pressure of 150 kPa. Cholesterol in the tissue sample was calculated according to the amount of internal standard stigmastanol added (*17*).

Determination of Policosanol. The individual policosanols in either diet or fecal samples were derivated to its TMS-ether by a commercial TMS reagent (Sigma). Analysis of the policosanol TMS-ether derivative was also performed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Inc.) in a Shimadzu GC-14B GLC equipped with a flame ionization detector (Shimadzu).

Quantification of Fecal Policosanol and Neutral and Acidic Sterols. Individual fecal neutral and acidic sterols were quantified as previously described (*17*). In brief, stigmasterol (0.5 mg in 1 mL of chloroform) as an internal standard for neutral sterols was added to a fecal sample (300 mg). The sample was then saponified using 9 mL of 1 mol/L NaOH in 90% ethanol containing 0.5 mg hyodeoxycholic acid in 2 mL of 1 N NaOH as an internal standard for acidic sterols (Sigma). The total policosanol and neutral sterols were extracted using 8 mL of cyclohexane and were then converted to their corresponding TMS-ether derivatives for GLC analysis.

After the cyclohexane extraction, 1 mL of 10 mol/L NaOH was added to the remaining aqueous layer and heated at 120 °C for 3 h. After the mixtures were cooled, 1 mL of distilled water and 3 mL of 3 N HCl were added followed by extraction with 7 mL of diethyl ether twice. The diethyl ether layers were then pooled followed by adding 2 mL of methanol, 2 mL of dimethoxypropane, and 40 μL of concentrated HCl (12 mol/L). After the solutions stood overnight at room temperature, the solvents were dried down and the acidic sterols were similarly converted to their TMS-ether derivatives at 60 °C for GLC analysis.

LDL Isolation and Oxidation. Blood was obtained from Hong Kong Red Cross Blood Transfusion Service (Kowloon, Hong Kong). To prevent the lipoprotein from oxidative modification, EDTA (2.7 mmol/L) and NaN_3 (7.7 mmol/L) solutions were added before LDL was isolated from serum according to the method previously described (*18*). The protein content of isolated LDL was determined using Lowry's method (*19*).

The oxidation of LDL was conducted as previously described (*18*). In brief, the stock LDL fraction (5 g protein/L) was dialyzed against 100 volumes of the degassed dialysis solution (pH 7.4) containing 0.01 mol/L sodium phosphate, 9 g/L NaCI, 10 μmol/L EDTA, and 7.7 mmol/L NaN_3 in dark for 24 h. The dialysis solution was changed at least four times. Then, the dialyzed LDL was diluted to 250 mg protein/L with 0.01 mol/L sodium phosphate buffer (pH 7.4). For the control incubation tubes, 0.4 mL of LDL (250 mg/L) was mixed with 50 μL of 50 μmol/L $CuSO_4$ solution and 50 μL of 0.01 mol/L sodium phosphate buffer (pH 7.4) and incubated at 37 °C for up to 40 h. For the experimental tubes, 0.4 mL of LDL (250 mg protein/L) was preincubated with 50 μL of individual policosanol in dimethyl sulfoxide. Then, 50 μL of 50 μmol/L $CuSO_4$ solution was added to initiate the oxidation, followed by incubation at 37 °C for up to 40 h. The oxidation was then stopped by the addition of 25 μL of 27 mmol/L EDTA and cooled at 4 °C. The degree of LDL oxidation was monitored by measuring the production of thiobarbituric acid reactive substances (TBARS) as previously described (*18*). The LDL-incubated tubes were immediately added with 2 mL of 0.67% thiobarbituric acid and 15% trichloroacetic acid in 0.1 N HCL solution. The incubation mixture was then heated at 95 °C for 1 h, cooled on ice, and centrifuged at 1000*g* for 20 min. TBARS were then determined by measuring the absorbance at 532 nm. The calibration was done using a malondialdehyde (MDA) standard solution prepared from tetramethoxylpropane. The value of TBARS was expressed as nmol MDA/mg LDL protein.

Free Radical Scavenging Assay. The anti-free radical activity of each policosanol was also examined as previously described (*20*). In brief, 0.75 mL of methanol containing 60 μM each policosanol was mixed in a test tube with 2.5 mL of methanol containing 75 μM 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a stable free radical and has a typical absorbance at 517 nm. The reaction mixture was maintained in dark at room temperature for 90 min, and the absorbance at 517 nm was then recorded. The free radical scavenging activity was calculated as the following equation:

$$\text{scavenging activity (\%)} = [A_a - (A_b - A_c)]/A_a \times 100$$

where A_a is the absorbance of the incubation DPPH solution without the addition of the tested policosanol; A_b is the absorbance of the incubation mixture containing the tested policosanol and DPPH; and A_c is the absorbance of the blank solution without DPPH.

Statistics. Data are expressed as means ± standard deviation. Where applicable, analysis of variance (ANOVA) was used to statistically evaluate significant differences among the control, P0.38, P0.75, and P1.50 groups using Sigmastat (Jandel Scientific Software, San Rafael, CA). Subsequently, Student's t-test was used to compare differences between any two groups. Differences were considered significant when $p < 0.05$.

Animal Ethics. The protocol described in the present study was approved by The Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

Table 2. Body Weight, Organ Weight, and Food Intake in Hamsters Fed the Control Diet and the Experimental Diets Supplemented with Varying Amounts of Policosanol[a]

	control	P0.38	P0.75	P1.5
initial body weight (g)	107.0 ± 9.5	107.5 ± 8.2	105.0 ± 9.1	106.0 ± 6.6
final body weight (g)	120.2 ± 10.3	118.2 ± 6.5	116.8 ± 11.8	118.4 ± 6.2
food intake (g/day)	10.3 ± 0.8	10.5 ± 0.8	10.9 ± 0.7	11.2 ± 0.8
liver (g)	3.90 ± 0.51	3.87 ± 0.29	3.81 ± 0.78	3.94 ± 0.45
heart (g)	0.46 ± 0.04	0.46 ± 0.03	0.44 ± 0.04	0.44 ± 0.05
kidney (g)	0.97 ± 0.08	0.98 ± 0.10	0.99 ± 0.11	0.97 ± 0.06
brain (g)	0.99 ± 0.41	1.00 ± 0.03	0.99 ± 0.06	1.01 ± 0.04
adipose tissues	1.64 ± 0.41	1.46 ± 0.33	1.59 ± 0.33	1.58 ± 0.36

[a] Values are means ± SD.

Table 3. Changes in Serum TC, Total Triacylglycerols (TG), HDL-C, and Non-HDL-C/HDL-C in Hamsters Fed the Control Diet and the Experimental Diets Supplemented with Varying Amounts of Policosanol[a]

	control	P0.38	P0.75	P1.5
		week 0		
TC (mg/dL)	119.6 ± 11.3	119.5 ± 9.4	119.7 ± 10.8	119.2 ± 14.2
HDL-C (mg/dL)	48.3 ± 5.2	49.5 ± 7.3	47.5 ± 5.6	48.9 ± 7.5
non-HDL-C (mg/dL)	71.3 ± 21.3	70.0 ± 26.5	72.1 ± 27.3	70.3 ± 28.9
TG (mg/dL)	230.2 ± 88.6	211.9 ± 45.6	231.4 ± 62.6	241.8 ± 55.9
non-HDL-C/HDL-C	1.5 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.3
		week 2		
TC (mg/dL)	164.2 ± 17.7	164.4 ± 25.6	163.8 ± 25.9	167.2 ± 31.9
HDL-C (mg/dL)	49.5 ± 5.5	56.0 ± 8.9	58.2 ± 6.6	62.2 ± 6.6
non-HDL-C (mg/dL)	93.8 ± 27	108.4 ± 28.6	105.6 ± 29.3	105.0 ± 30.8
TG (mg/dL)	177.3 ± 41.6	245.1 ± 58.7	263.7 ± 69.8	224.9 ± 72.5
non-HDL-C/HDL-C	1.9 ± 0.5	2.0 ± 0.7	1.9 ± 0.7	1.7 ± 0.5
		week 4		
TC (mg/dL)	193.9 ± 49.5 a	153.3 ± 21.9 b	145.1 ± 19.9 b	149.6 ± 22.4 b
HDL-C (mg/dL)	51.3 ± 5.4 b	56.7 ± 9.0 ab	56.7 ± 4.8 a	59.9 ± 8.0 a
non-HDL-C (mg/dL)	142.7 ± 48.0 a	96.7 ± 18.6 b	88.4 ± 21.3 b	80.8 ± 34.9 b
TG (mg/dL)	286.9 ± 89.7	217.8 ± 66.6	284.9 ± 106.1	270.8 ± 75.0
non-HDL-C/HDL-C	2.8 ± 0.9 a	1.7 ± 0.4 b	1.6 ± 0.4 b	1.5 ± 0.4 b
		week 6		
TC (mg/dL)	186.4 ± 28.1 a	157.5 ± 15.6 b	151.1 ± 14.6 b	147.1 ± 16.7 b
HDL-C (mg/dL)	52.8 ± 7.3 b	56.5 ± 3.8 ab	56.6 ± 2.0 ab	58.0 ± 3.4 a
non-HDL-C (mg/dL)	133.6 ± 23.4 a	101.0 ± 13.6 b	95.5 ± 15.0 b	89.2 ± 18.7 b
TG (mg/dL)	239.2 ± 74.5	229.1 ± 64.5	237.4 ± 41.1	237.8 ± 56.5
non-HDL-C/HDL-C	2.6 ± 0.4 a	1.8 ± 0.2 b	1.7 ± 0.3 b	1.6 ± 0.4 b

[a] Values are means ± SD. Means in a row for a given week with different letters differ significantly; $p < 0.05$.

RESULTS

Food Intake and Body Weight. The policosanol sample used in the present study was 99% purity and contained 52% 1-octacosanol (C28) and 42% 1-triacontanol (C30). Four diets contained 0 (control), 0.38 (P0.38), 0.75 (P0.75), and 1.5 g (P1.50) of policosanol per kg diet, respectively (**Table 1**). The hamsters were fed one of the four diets for 6 weeks. No significant differences in body weight gain were observed although the P0.75 group had a smaller average body weight as compared with the other three groups (**Table 2**). Similarly, there were no significant differences in food intakes among the four groups. The organ weights were similar among the four groups.

Serum TC, HDL-C, TG, and Non-HDL-C/HDL-C. The change in serum lipid profile was first observed within each group (**Table 3**). When four groups of hamsters were placed on their diets containing high cholesterol (0.1%, w/w) but varying amounts of policosanol for 6 weeks, their serum TC level was elevated significantly in the control (from 119.6 to 186.4 mg/dl), although it was elevated to a lesser extent in the policosanol-supplemented hamsters (P0.38, from 119.5 to 157.5; P0.75, from 119.7 to 151.1; and P1.5, from 119 to 147. 1 mg/dL). The elevation in serum TC was mainly associated with an increase in non-HDL-C levels without significantly affecting HDL-C.

The significant differences among the four groups were noticed. Four groups had similar levels of serum TC, HDL-C, and TG at the beginning of the experiment and the end of week 2 (**Table 3**). At the end of week 4, serum TC and non-HDL cholesterol levels of three experimental groups started to be significantly lower as compared with those of the control. In contrast, serum HDL-C levels started to be significantly higher in P0.75 and P1.50 groups as compared with that of the control, leading to a lower ratio of non-HDL-C to HDL-C (**Table 3**). No significant differences in serum TC, HDL-C, non-HDL-C, and TG were observed among the three experimental groups at the end of week 4. At the end of week 6, a similar trend was observed. Policosanol at 0.38, 0.75, and 1.50 g kg^{-1} diet led to a significant decrease in serum TC, non-HDL-C, and ratio of non-HDL-C to HDL-C. Only the P1.50 group had a HDL-C level significantly elevated as compared with the control group. Regarding serum TG level, individual variations were so significant so that no statistical difference was observed among the four groups throughout the entire experiment.

Liver, Heart, and Kidney Cholesterol. Incorporation of policosanol into diet had no significant effect on cholesterol levels in the liver, heart, and kidney (**Table 4**). No difference in adipose tissue cholesterol levels was seen among the control and three experimental groups.

Fecal Output of Policosanol, Acidic Sterols, and Neutral Sterols. Total fecal neutral sterols refer to a sum of cholesterol, coprostanol, dihydrocholesterol, campersterol, and β-sitosterol (**Table 5**). The total fecal neutral sterols were significantly elevated only in the P0.75 group but not in the P0.38 and P1.50 groups. There was no significant difference in the excretion of

Table 4. Cholesterol Content (mg/g) of the Liver, Heart, and Kidney in Hamsters Fed the Experimental Diets Supplemented with Varying Amounts of Policosanol[a]

	control	P0.38	P0.75	P1.5
liver	14.2 ± 2.3	13.9 ± 1.4	14.5 ± 1.7	13.6 ± 1.4
heart	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
kidney	4.0 ± 0.4	4.0 ± 0.3	3.8 ± 0.3	3.9 ± 0.3
adipose tissue	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.2

[a] Values are means ± SD.

Table 5. Fecal Output of Policosanol, Acidic Sterols, and Neutral Sterols (mg/Hamster/Day)[a]

	control	P0.38	P0.75	P1.50
coprostanol	0.90 ± 0.19 b	1.04 ± 0.17 ab	1.38 ± 0.35 a	0.98 ± 0.35 ab
cholesterol	0.55 ± 0.02	0.42 ± 0.06	0.48 ± 0.10	0.58 ± 0.31
dihydrocholesterol	0.46 ± 0.06	0.50 ± 0.04	0.58 ± 0.14	0.49 ± 0.48
β-sitosterol	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.17
total neutral sterols	1.96 ± 0.35 b	2.00 ± 0.31 ab	2.48 ± 0.48 a	2.10 ± 0.17 ab
lithocholic	0.33 ± 0.03 c	0.41 ± 0.06 b	0.49 ± 0.05 a	0.44 ± 0.10 ab
chenodeoxycholic acid	0.10 ± 0.02 b	0.16 ± 0.06 ab	0.17 ± 0.03 a	0.16 ± 0.07 ab
cholic acid	0.05 ± 0.01 b	0.09 ± 0.04 ab	0.11 ± 0.02 a	0.13 ± 0.08 a
ursodecholic acid	0.10 ± 0.05	0.08 ± 0.04	0.16 ± 0.04	0.16 ± 0.03
total acidic sterols	0.59 ± 0.10 c	0.74 ± 0.11 bc	0.93 ± 0.08 a	0.82 ± 0.11 ab
octacosanol (C28)	0	0.90 ± 0.22 c	2.25 ± 0.55 b	4.29 ± 0.55 a
triacontanol (C30)	0	0.48 ± 0.11 c	1.29 ± 0.27 b	2.45 ± 0.28 a
total policosanols	0	1.44 ± 0.33 c	3.64 ± 0.84 b	6.95 ± 0.83 a

[a] Values are means ± SD. Means at the same row with different superscript letters differ significantly; $p < 0.05$.

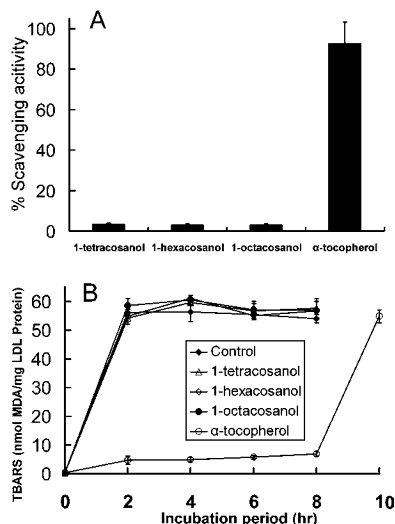

Figure 1. (**A**) Free radical scavenging activity of individual policosanol (60 μM). DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a stable free radical. Data are expressed as means ± SD of $n = 5$ samples. (**B**) Effect of individual policosanol (60 μM) on production of thiobarbituric acid reactive substances (TBARS) in Cu^{2+}-mediated oxidation of human LDL. The LDL (100 mg protein/L) was incubated in sodium phosphate buffer (pH 7.4) containing 5 μmol/L $CuSO_4$. The oxidation was conducted at 37 °C. Data are expressed as means ± SD of $n = 5$ samples.

cholesterol except for the P0.38 group that had a lower fecal cholesterol as compared with the control group. Regarding coprostanol, the P0.75 group had a greater excretion than the control group. No difference among the four groups was observed for the excretion of dihydrocholesterol and β-sitosterol.

The acidic sterols measured included cholic, chenodeoxycholic, lithocholic, and ursodeoxycholic acids (**Table 5**). In general, the consumption of policosanol increased excretions of total bile acids by 25−57% (**Table 5**). However, excretion of cholic, chenodeoxycholic, lithocholic, and ursodeoxycholic acids was not dose-dependent on policosanol. It was found that excretion of these bile acids was greater in the P0.75 group as compared with the other two tested groups.

The excretion of fecal policosanol was proportional to its amount in diet. The control group had no policosanol excreted while the three tested groups had 1.44, 3.64, and 6.95 mg of policosanol excreted per day per hamster.

Antioxidant Activity of Policosanols. The anti-LDL oxidation activity of policosanol was compared with α-tocopherol. The results demonstrated that policosanol had no anti-LDL oxidation. As shown in **Figure 1**, LDL was rapidly oxidized in LDL with the addition of 60 μM each policosanol but the induction time (lag time) for α-tocopherol was at least 8 h. Similarly, policosanol had no or weak scavenging activity on DPPH free radicals as compared with α-tocopherol (**Figure 1**). Three policosanols tested had only 3% DPPH free radical scavenged while α-tocopherol had DPPH free radical scavenged by 94%.

DISCUSSION

The present study demonstrated clearly that policosanol possessed a favorable effect on serum lipids. Supplementation of policosanol in the diet at the levels of 0.38−1.5 g/kg diet (equivalent to 4−16 mg/hamster/day) had serum TC levels lowered by 21−25% at week 4 and by 15−21% at week 6. In contrast, supplementation of policosanol had HDL-C elevated by 10−16.8% at week 4 and by 7−9.8% at week 6 although the increase was statistically significant only if 1.5 g/kg diet policosanol was formulated into the diet. The present results are in agreement with those reported in recent human studies. Castano et al. (*2*) found that serum TC was reduced by 12−16% while HDL-C was elevated by 14−29% in type II hypercholesterolemia patients given 5−10 mg of policosanol per day for 12 weeks. In the study conducted by Arruzazabala et al. (*21*), policosanol at doses of 20−40 mg/day for 30 days could reduce serum TC by 12% but it increased HDL-C only by 5% in dyslipidaemic patients. The reduction of serum TC by 10−12% and elevation of HDL-C by 9−15% was also observed in healthy volunteers who were given 5−10 mg/day for 8 weeks (*22*). In a long-term study, an oral dose of 5 mg of policosanol with the evening meal for 3 years significantly maintained serum TC levels 20.1% lower and HDL-C levels 24.6% higher in obese patients with type II hypercholesterolemia (*23*). In male monkeys (*Macaca arctoides*) given 0.25, 2.5, and 25 mg/kg policosanol for 54 weeks, a similar effective reduction in serum TC and LDL-C was observed (*5*). In a study using beagle dogs to test the toxicity of policosanol, it was found that policosanol was associated with a 20% reduction in serum TC. When rabbits were used as a hypercholesterolaemia model, administration of policosanol lowered both serum TC and LDL-C levels. The present study was the first to demonstrate that policosanol could also reduce serum TC and increase HDL-C levels in hamsters given a moderate cholesterol diet. However, the result is in disagreement with that of Wang et al (*9*), who did not find any effect of policosanol supplementation on serum TC in hamsters given a 0.25% cholesterol diet. We have no explanation for this discrepancy. Perhaps, the cholesterol level (0.25%) in the study by Wang et al (*9*) was too high to demonstrate any effect of policosanol on blood cholesterol level as compared with the 0.10% cholesterol diet used in the present study.

It should also be pointed out that the cholesterol-lowering effect of policosanol under the present experimental conditions was not dose-dependent. As shown in **Table 3**, no statistical difference among the three policosanol-supplemented groups was seen at the end of weeks 4 and 6. Perhaps, 0.38 g of policosanol per kg diet (P0.38) used in the present study was already a dose that had reached a maximum effect, leading to no further cholesterol-lowering effect on serum lipid when policosanol was increased to 0.75 or 1.5 g per kg diet.

Policosanol has been demonstrated to reduce cholesterol synthesis by down-regulation of 3-hydroxy-3-methylglutary coenzyme A (HMG-CoA) reductase, thus lowering blood cholesterol (*10*, *11*). The present study was carried out further to demonstrate an additional mechanism by which policosanol could lower blood cholesterol. The present results showed that policosanol was able to increase the excretion of bile acids although a dose-dependent effect was not associated with that of P1.5 group (**Table 5**). The major metabolites of cholesterol are bile acids. Greater excretion of fecal bile acids can lead to a lower level of blood cholesterol. Microbial conversion of primary bile acids leads to production of secondary bile acids. In this regard, the bile acids should be considered as a whole rather than as individuals. The blood cholesterol is maintained in a steady balance in which the rate of entry of cholesterol into the blood is equal to the removal of cholesterol from the blood. A lowered cholesterol level indicates a shift in this balance, resulting from either a decrease in the rate of entry or an increase in the rate of removal. The present study found that greater excretion of acidic sterols was associated with consumption of policosanol. The consequence of greater excretion of

bile acids would lead to a greater conversion of cholesterol to bile acids in the liver, resulting in the entry of cholesterol into the liver from blood and leading to a reduction in blood cholesterol level.

Policosanol has been claimed to reduce susceptibility of LDL to oxidation (*22*). The present results did not prove this hypothesis and found that individual policosanol had no inhibition on human LDL oxidation in vitro. Menendez et al. (*22*) compared the susceptibility of LDL isolated from the subjects given orally 5 and 10 mg of policosanol per day with that isolated from the control subjects on placebo, finding that the lag phase of LDL oxidation was extended as compared with the placebo control. However, the subjects consumed their regular diets and no attempt was made to analyze individual variation in the composition of LDL in the study conducted by Menendez et al. (*22*). The present study used human LDL in vitro to eliminate the uncertainty of LDL composition and tested three pure policosanols rather than a mixture. The results clearly demonstrated that policosanol had no direct anti-LDL oxidation activity. If policosanol has anti-LDL oxidation in vivo, its action must be indirect by modifying the composition of LDL in a way that is resistant to oxidation.

In conclusion, policosanol produced a desirable effect on the blood lipid profile by decreasing TC and LDL-C while increasing HDL-C levels. Policosanol was shown previously to reduce cholesterogenesis by inhibition on HMG-CoA reductase (*10*, *11*) and increase the clearance of LDL-cholesterol by increasing LDL binding, uptake, and degradation (*12*). The present study explored an additional mechanism by which policosanol might inhibit absorption and increase excretion of acidic sterols. Policosanol is not an antioxidant at least in LDL and DPPH systems used in the present study.

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