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Very long chain fatty acids (policosanols) and phytosterols affect plasma lipid levels and cholesterol biosynthesis in hamsters

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Abstract

The aim of the current study was to examine the effects of very long chain fatty acids (VLCFA) alone at 2 dietary levels, or in combination of VLCFA at the lower level with lecithin (LT) or phytosterols (PS), on lipid profiles and cholesterol biosynthesis in hamsters. Seventy-five male Golden Syrian hamsters, weighing 100 to 120 g, were fed a regular rodent chow for 2 weeks before being randomly assigned into 5 groups of 15 animals each fed semisynthetic diets for 4 weeks. Group 1 was given a control diet that contained 0.25% cholesterol and 5% fat with a polyunsaturated to saturated fatty acids ratio of 0.4. Groups 2 to 5 were fed the control diet and given 25 mg/kg BW per day of VLCFA (Licowax) (VLCFA25), 50 mg/kg BW per day of VLCFA (VLCFA50), 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of LT (VLCFA25/LT), and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of PS (Cholestatin, VLCFA25/PS), respectively. Results showed that HDL-cholesterol (HDL-C) levels were not changed by VLCFA25, although increased by VLCFA50 ($P < .05$) relative to control. Total cholesterol (T-C) and non-HDL-C levels were not affected by VLCFA25 and VLCFA50 as compared with control. VLCFA25/LT had higher ($P < .02$) T-C and HDL-C levels than any other treatments and increased ($P < .05$) liver weight relative to control. In contrast, VLCFA25/PS reduced T-C ($P = .0004$) and non-HDL-C ($P = .007$) without effect on HDL-C levels compared with control. Triglyceride levels were not affected by any treatment. Cholesterol biosynthesis rate was higher ($P < .05$) in animals fed VLCFA25 and VLCFA50 than those fed control or VLCFA25/LT or VLCFA25/PS. Results suggest that PSs can decrease total and non-HDL-C cholesterol, whereas VLCFA may increase HDL-C in hamsters.

1. Introduction

Policosanols are mixtures of very long chain fatty alcohols isolated from sugar cane wax, bee wax, and other plant waxes [1]. Major components of policosanols are octacosanol, triacontanol, and hexacosanol, with tetracosanol, heptacosanol, nonacosanol, dotriacontanol, and tetratriacontanol existing as minor components [1,2]. Over the last decade, policosanols have been studied, mainly in Cuba, for their cholesterol-lowering effect [3-7]. Varady et al [8] suggested that although significant reductions in cholesterol levels have been observed after policosanol consumption, further studies are warranted to investigate the effect and mechanisms of the cholesterol-lowering action of policosanols, particularly in different ethnic populations and animal models.

As policosanols are oxidized after ingestion to fatty acids through β-oxidation [9-13], very long chain fatty acids (VLCFA) are implicated as the active form of policosanols influencing cholesterol metabolism. Supportive data have been reported by others who fed rats with total hydrogenated fish oil [14] or rabbits with policosanols isolated from sugar cane wax [15]. Our previous study in hamsters did not show significant reductions in plasma lipids after feeding policosanols isolated from sugar cane [16]. However, hamsters fed a mixture of policosanols (25 mg/kg BW per day) and the corresponding VLCFA (25 mg/kg BW per day) derived from rice wax showed 3% to 8% lower, although not significant, in total, LDL- and HDL-cholesterol levels relative to those fed the policosanols alone [16]. In vitro studies suggest that policosanols/VLCFA reduce cholesterol by inhibiting biosynthesis [17,18]. This finding has been supported by other studies [5,17,19,20]. However, this observation has not been

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supported by a recent study in hamsters [16]. It is unknown whether the discrepancies between a recent hamster study [16] and studies in other animals or humans [1-8] were caused by animal species or the dosage, composition, and delivery form of the test materials.

Phytosterols (PS) have now been unquestionably established as capable of lowering plasma cholesterol levels [21-27]; an inhibition of cholesterol absorption is partially compensated for by a reciprocal increase in cholesterol biosynthesis rate [23,24]. If VLCFA can inhibit cholesterol biosynthesis, the combination of VLCFA with PS may produce an additive effect in reducing cholesterol levels by suppressing both absorption and biosynthesis. Therefore, the objectives of the current study were to determine (1) the effect of VLCFA alone at 2 different levels on plasma cholesterol levels and biosynthesis; (2) whether lecithin (LT) can increase the solubility of VLCFA and thus enhance the lipid-altering effect of VLCFA; and (3) whether VLCFA and PS act additively to change the circulating cholesterol levels by suppressing both absorption and biosynthesis.

2. Materials and methods

2.1. Animals and diets

Seventy-five male Golden Syrian hamsters (Charles River Laboratories, Wilmington, Mass) weighing 100 to 120 g were housed in individual steel cages and subjected to a 12-hour light/dark cycle. Animals were given free access to regular rodent chow (JE Mondou, Anjou, QC, Canada) and water for 2 weeks. Hamsters were weighed and randomly assigned into 5 groups of 15 animals each before commencing the study. Experimental diets were provided in accordance with AIN-76 formulation with modification [16]. Group 1 was given a moderate atherogenic control diet that contained 0.25% cholesterol and 5% fat (Table 1). The polyunsaturated fatty acids to saturated fatty acids ratio of the control was 0.4. Groups 2 to 5 were fed the control diet and given 25 mg/kg BW per day of VLCFA (Licowax), 50 mg/kg BW per day of VLCFA, 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of LT, and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of PSs (Cholestatin), respectively. The diets were accordingly designated control, VLCFA25, VLCFA50, VLCFA25/LT, and VLCFA25/PS. All the test materials were provided by Degussa BioActives (Champaign, Ill, USA). Licowax is a VLCFA mixture derived from montan wax and comprises 69% VLCFA (8% C24:0, 13% C26:0, 18% C28:0, 18% C30:0, 7% C32:0, 3% C34:0, 1% C36:0, and 1% others with the carbon chain > C36). Cholestatin contains 85% sterols (40% β-sitosterol, 20% campesterol, 11% stigmasterol, and 0.3% brassicasterol, respectively). Licowax and LT were dissolved in the oil component of the diets by rapid heating to 70 °C to 80 °C by microwave. Phytosterols in finely milled form were suspended in the oil first by heating at 55 °C and then mixed into diets. Diets were prepared weekly and stored at −20 °C.

Table 1
Composition of control diet fed to hamsters

Ingredients[a]	g/kg
Casein	200
Corn starch	280
Sucrose	363
Beef tallow/safflower oil	50
Cellulose	49.55
DL-Methionine	5
Mineral mix[b]	40
Vitamin mix[c]	10
Choline bitartrate	2
Cholesterol	0.25
Butylated hydroxytoluene	0.2

[a] All ingredients were purchased from ICN Pharmaceuticals, Inc (Costa Mesa, Calif).

[b] Mineral mix contains (per kilogram) 500 g calcium phosphate dibasic, 74 g sodium chloride, 220 g potassium citrate monohydrate, 52 g potassium sulfate, 24 g magnesium oxide, 3.5 g manganese carbonate (43%-48% Mn), 6 g ferric citrate (16%-17% Fe), 1.6 g zinc carbonate (70% ZnO), 0.3 g cupric carbonate (53%-55% Cu), 0.01 g potassium iodate, 0.01 g sodium selenite, 0.55 g chromium potassium sulfate, and 118 g finely powdered sucrose.

[c] Vitamin mix contains (per kilogram) 600 mg thiamine hydrochloride, 600 mg riboflavin, 700 mg pyridoxine hydrochloride, 3 g nicotinic acid, 1.6 g D-calcium pantothenate, 200 mg folic acid, 20 mg D-biotin, 1 mg cyanocobalamin (vitamin B_{12}), 1.6 g retinyl palmitate (vitamin A) premix (250 000 IU/g), 20 g DL-α-tocopherol acetate (250 IU/g), 250 mg cholecalciferol (vitamin D_3, 400 000 IU/g), 5 mg menaquinone (vitamin K_2), and 973 g finely powdered sucrose.

Hamsters were fed the experimental diets for 4 weeks. Food intake was recorded daily and animals were weighed weekly. Two hours before sacrifice on day 28, animals were given 0.5 mL of deuterium oxide (CDN Isotopes, Montreal, Canada) by intraperitoneal injection and foods were removed immediately. Two hours after the injection, animals were anesthetized by carbon dioxide inhalation, and blood samples were collected by decapitation. Plasma and red blood cells were separated and stored at −80 °C. The liver, heart, kidney, and brain were carefully removed and weighed. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee, McGill University, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Plasma lipid analysis

Plasma total cholesterol (T-C), HDL cholesterol (HDL-C), and triglyceride (TG) levels were measured in triplicate using commercial enzymatic kits (Boehringer Mannheim Corporation, Indianapolis, Ind). Measurement of HDL-C in plasma was done after the precipitation of apo B–containing lipoproteins. Because the Friedewald equation may not have been applicable to hamsters [28], non-HDL-C (VLDL + IDL + LDL cholesterol) instead of LDL-C was used. The non-HDL-C was calculated by subtracting HDL-C from T-C.

2.3. Cholesterol biosynthesis measurement

Circulating cholesterol was selected for sampling as it is both readily accessible and is a part of the rapid-turnover

pool that also comprises hepatic and intestinal sterol [29]. Within the plasma compartment, although deuterium uptake occurs in both free and ester pools, incorporation into the total free compartment exceeds that into ester by twofold [30]. The faster uptake into free sterol indicates its role as the primary compartment to receive newly synthesized sterol. Of the available options, free plasma cholesterol is thus likely to be most sensitive to perturbation in cholesterogenesis [31]. The free cholesterol component of this pool equilibrates quickly between plasma and other subpools [29,32]. Erythrocytes do not synthesize cholesterol [32]; thus, sterol in this subcompartment is obtained from the plasma. Erythrocyte cholesterol, found solely in free form, enables the use of smaller blood samples [31]. Model considerations have been previously described [31,33]. The kinetics model in a period of 24 hours post–oral dose of deuterium oxide that was used to calculate cholesterol biosynthesis has been extensively validated against sterol balance [34], mass isotopomer distribution analysis [35], and sterol precursor levels [36,37]. In animals, a period of 1 or 2 hours has been used to measure cholesterol biosynthesis rate [26,38]. It has been demonstrated that deuterium enrichment over the 24-hour cholesterol biosynthesis measurement period is relatively linear [31]. On this basis, any 2-hour fraction of this period should provide a good estimate of synthesis extrapolated across the entire day.

Approximately 0.2 g red blood cells were placed into a 15-mL Pyrex (Corning Glass Works, Corning, NY) tube with 4 mL methanolic KOH and heated for 2 hours at 95 °C. After cooling to room temperature, lipids were extracted with 4 mL petroleum ether and 2 mL water, vortexed, centrifuged for 15 minutes at 1500 rpm, and solvent was removed. The extraction was repeated twice. Solvent layers were combined and thoroughly dried under nitrogen. Neutral sterols were converted to trimethylsilyl (TMSi) ethers by adding 2 mL TMSi reagent (pyridine-hexamethyldisilazan-trimethylchlorosilane, 9:3:1, vol/vol/vol) as reported by Lutjohann et al [39]. After heating the samples at 70 °C for 1 hour, the silylating reagent was evaporated under nitrogen. The TMSi derivatives were dissolved in 1 mL of hexane and 0.5 μL was injected into the gas chromatograph mass spectrometer.

2.4. Gas chromatograph–mass spectrometry

Gas chromatograph–mass spectrometry was carried out using selective ion monitoring mode on an Agilent 6890 gas chromatograph fitted with an Agilent 7683 automatic sample injector and interfaced to an Agilent 5973 mass spectrometer (Agilent Technologies, Wilmington, Del, USA). The TMSi derivatives of neutral sterols were separated on a 30-m fused silica capillary column (HP SAC-5, inner diameter 0.25 mm; Supelco, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The oven temperature was set to an initial value of 250 °C for 1 min followed by a temperature program of increasing at 1.3 °C/min to the final of 280 °C. The temperature of the transfer line was kept at 280 °C. Electron impact ionization was applied with 70 eV. Ions of *m*/*z* 329, 330, and 331 were monitored for quantification of cholesterol, D_1-cholesterol and D_2-cholesterol, respectively. The cholesterol biosynthesis rate was examined by measuring the ratio between the counts of ions representing the most abundant synthesized cholesterol with 1 (*m*/*z* 330) and 2 deuterium (*m*/*z* 331) atoms and the natural cholesterol (*m*/*z* 329). The mass ratios were then converted into deuterium/hydrogen ratios. A mean average value for the deuterium enrichment of free cholesterol found in humans and animals situated in the west of Montreal (−220 relative to the Standard Mean Ocean Water working standard) was converted into deuterium/hydrogen ratio and used as the baseline for hamster cholesterol. The increase in the ratio of deuterium/hydrogen in free cholesterol 2 hours after deuterium injection, relative to the baseline, was taken to represent the amount of newly synthesized cholesterol and was expressed as a percent rate of the body cholesterol pool.

2.5. Statistical analysis

All data were tested for normality and a natural log transformation was performed for the data of triglyceride. As each treatment diet contained VLCFA, the main treatment effects were analyzed by 1-way analysis of variance (ANOVA) using the general linear model procedure of SAS software (SAS Institute, Inc, Cary, NC) [40]. Significant

Table 2
Food intake, body weight, and tissue weight of hamsters fed diets with very long chain fatty acids alone and in combination with either LT or PSs for 4 weeks*

Diet**	Food intake (g/d)	Body weight (g)	% of body weight			
			Liver	Heart	Kidney	Brain
Control	7.8 ± 0.5	135 ± 10	2.06 ± 0.29^{b,c}	0.41 ± 0.03	0.77 ± 0.04	0.76 ± 0.05
VLCFA25	7.9 ± 0.8	137 ± 15	2.24 ± 0.24^{a,b}	0.40 ± 0.02	0.80 ± 0.05	0.77 ± 0.07
VLCFA50	7.8 ± 0.7	136 ± 15	2.19 ± 0.29^{a,b}	0.39 ± 0.03	0.77 ± 0.06	0.76 ± 0.08
VLCFA25/LT	7.9 ± 0.8	139 ± 14	2.33 ± 0.19^{a}	0.40 ± 0.03	0.81 ± 0.05	0.74 ± 0.07
VLCFA25/PS	7.9 ± 0.7	135 ± 13	1.96 ± 0.33^{c}	0.40 ± 0.03	0.77 ± 0.03	0.75 ± 0.06

Values with different superscripts within a column are significantly different ($P < .05$).

* Values are means ± SD (n = 15).

** VLCFA25, treatment given a control diet and 25 mg/kg BW per day of very long chain fatty acids (VLCFA) (Licowax); VLCFA50, treatment given a control diet and 50 mg/kg BW per day of VLCFA; VLCFA25/LT, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of LT; VLCFA25/PS, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of PSs.

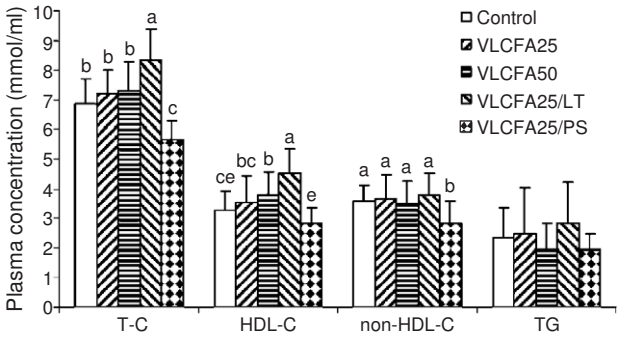

Fig. 1. Effects of very long chain fatty acids and phytosterols on plasma lipid profiles in hamsters fed corn starch-cheese-sucrose–based moderately atherogenic diet for 4 weeks. Data across treatment groups were analyzed by one-way ANOVA. Where a significance level of less than .05 was achieved, differences between group means were evaluated using the method of PDiff matrix of least squares means test. Values are means ± SD (n = 15). For each parameter, bars with different superscripts are different ($P < .05$). T-C, total cholesterol; HDL-C, HDL cholesterol; non-HDL-C, VLDL + IDL + LDL cholesterol; TG, triglyceride. VLCFA25, treatment given a control diet and 25 mg/kg BW per day of VLCFAs (Licowax); VLCFA50, treatment given a control diet and 50 mg/kg BW per day of VLCFA; VLCFA25/LT, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of lecithin; VLCFA25/PS, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of phytosterols.

differences among dietary treatments were analyzed by the method of PDiff Matrix of least squares means after a significant main effect by 1-way ANOVA [41]. Significance level was set at $P < .05$. Data were expressed as means ± SD.

3. Results

3.1. Body weight and food intake

Data of food intake and body weight are presented in Table 2. Body weight and food intake were not affected by the dietary treatments during the 4-week feeding period. The intake of VLCFA was 24.8 ± 2.1, 49.8 ± 3.0, 23.6 ± 2.7, and 24.4 ± 2.5 mg/kg BW per day for hamsters fed diets VLCFA25, VLCFA50, VLCFA25/LT, and VLCFA25/PS, respectively. Lecithin intake was 979.2 ± 74.9 mg/kg BW per day for the VLCFA25/LT group and PS intake was 1011.8 ± 60.3 mg/kg BW per day for the VLCFA25/PS group.

3.2. Lipid profiles

The effects of dietary treatments on plasma lipid profiles are presented in Fig. 1. Hamsters fed VLCFA25/LT had increased T-C levels compared with control ($P < .0001$) and any other treatment ($P < .002$). In contrast, VLCFA25/PS reduced T-C levels relative to control ($P = .0004$) and the other treatments ($P < .0001$). Diet VLCFA50 elevated ($P < .05$) HDL-C in comparison with control and VLCFA25/PS. A further elevation ($P < .0001$) in HDL-C was found in animals fed VLCFA25/LT, which showed higher ($P < .02$) HDL-C levels than any other treatment. The VLCFA25/PS did not alter HDL-C levels compared with control, but produced lower HDL-C levels than VLCFA25 ($P = .009$), VLCFA50 ($P = .0004$), and VLCFA25/LT ($P < .0001$), respectively. Animals fed VLCFA25/PS also showed lower ($P < .02$) levels of non-HDL-C than those fed the other diets, with no difference observed among the animals fed the other diets. There were no differences in T-C levels among VLCFA25, VLCFA50, and control groups. The levels of HDL-C did not differ between VLCFA25 and control, or between VLCFA25 and VLCFA50. The ratio of T-C to HDL-C was similar among the treatment groups (Fig. 2). Triglyceride levels were not influenced by any treatment (Fig. 1).

3.3. Cholesterol biosynthesis rate

The results are summarized in Table 3. The estimated amount of newly synthesized cholesterol was increased by VLCFA25 ($P < .0001$) and VLCFA50 ($P = 0.002$) compared with control. Hamsters fed VLCFA25 had higher cholesterol synthesis rate than those fed VLCFA25/LT ($P = .0002$) and VLCFA25/PS ($P = .007$). Hamsters fed VLCFA50 had higher ($P < .001$) cholesterol synthesis rate than those fed VLCFA25/LT. No differences were observed among control, VLCFA25/LT, and VLCFA25/PS.

3.4. Tissue weights

Weights of the liver, heart, kidney, and brain after the 4-week feeding period are presented in Table 2. Liver weight was not different among VLCFA25, VLCFA50, and control. There was no difference between control and VLCFA25/PS either, as well as among VLCFA25, VLCFA50, and VLCFA5LT, respectively. However, animals fed VLCFA25/LT had higher ($P < .05$) liver weight than those fed control or VLCFA25/PS. No differences

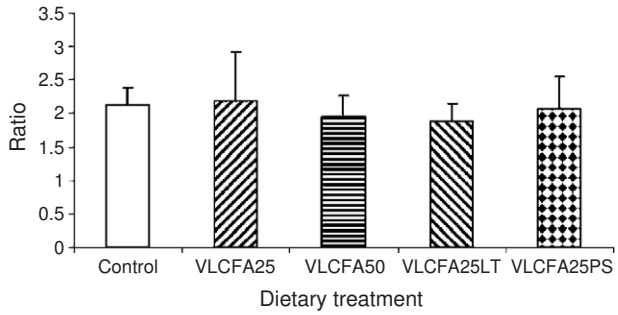

Fig. 2. Effects of very long chain fatty acids and phytosterols on the ratio of total cholesterol to HDL cholesterol in hamsters fed corn starch-cheese-sucrose–based moderately atherogenic diet for 4 weeks. Data across treatment groups were analyzed by one-way ANOVA. Where a significance level of less than .05 was achieved, differences between group means were evaluated using the method of PDiff of least squares means test. Values are means ± SD (n = 15). T-C, total cholesterol; HDL-C, HDL cholesterol; non-HDL-C, VLDL + IDL + LDL cholesterol. VLCFA25, treatment given a control diet and 25 mg/kg BW per day of VLCFAs (Licowax); VLCFA50, treatment given a control diet and 50 mg/kg BW per day of VLCFA; VLCFA25/LT, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of lecithin; VLCFA25/PS, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of phytosterols.

Table 3
The effects of dietary supplementation with very long chain fatty acids, LT, and PSs on cholesterol synthesis*

	Estimated amount of newly synthesized cholesterol (%)**
Control	6.64 ± 0.15^{b}
VLCFA25***	6.85 ± 0.25^{a}
VLCFA50	$6.86 \pm 0.22^{a,c}$
VLCFA25/LT	6.70 ± 0.40^{b}
VLCFA25/PS	$6.73 \pm 0.14^{b,c}$

Values with different superscripts are significantly different ($P < .05$).

* Data are presented as mean ± SD (n = 15).

** The increase in the ratio of deuterium/hydrogen in free cholesterol 2 hours after deuterium injection, relative to the baseline, was taken to represent the amount of newly synthesized cholesterol and was expressed as a percent rate of the body cholesterol pool.

*** VLCFA25, treatment given a control diet and 25 mg/kg BW per day of very long chain fatty acids (VLCFA) (Licowax); VLCFA50, treatment given a control diet and 50 mg/kg BW per day of VLCFA; VLCFA25/LT, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of LT; VLCFA25/PS, treatment given a control diet and 25 mg/kg BW per day of VLCFA + 1000 mg/kg BW per day of PSs.

were observed across different treatments in the weights of heart, kidney, and brain.

4. Discussion

The results of the present study showed that VLCFA increased HDL-C at the dose of 50 mg/kg BW per day, but exerted no effect on plasma T-C and non-HDL-C levels. The addition of LT to VLCFA diet produced a further elevation of HDL-C levels and, consequently, a significant increase in T-C levels as compared with control. The combination of VLCFA and PS decreased non-HDL-C and T-C levels, without effect on HDL-C levels relative to control. Levels of triglyceride were not changed by VLCFA or the combinations of VLCFA with LT or PS.

In agreement with previous studies [16,42-44], the present study did not show significant effects of VLCFA on body weight, food intake, or weight of heart, kidney, and brain. The combination of VLCFA with LT increased the liver weight. It is not known whether these statistical differences are biologically significant. The mechanism(s), biological effect, and safety of increased liver weight by VLCFA remain to be elucidated.

Our results contrast starkly to those of other reports. Oral administration of policosanols purified from sugar cane wax reduced serum cholesterol levels substantially in normocholesterolemic and hypercholesterolemic rabbits [3,5] and monkeys [4]. Human studies have demonstrated similar effects of policosanols in reducing blood cholesterol concentrations [1,2,6,7,45]. As the majority of these studies have been conducted in Cuba, it is important to assess policosanols and VLCFA in different regions with different animal models and ethnic populations. Wang et al [16] conducted a trial in hamsters, a well-established animal model for studying human lipid metabolism [27]. The results showed no significant change in cholesterol levels in comparison to control when hamsters were fed policosanols isolated from sugar cane at a dose of 25 mg/kg BW per day. As policosanols are oxidized after ingestion to VLCFA through β-oxidation in the body [9-13], VLCFA are suggested to be the active metabolites of policosanols for the cholesterol-lowering action [15,17,46,47]. This observation has been reevaluated in hamsters by feeding them with policosanols (25 mg/kg BW per day) and VLCFA (25 mg/kg BW per day) mixtures derived from rice wax [16]. Consistent reductions were observed in T-C, non-HDL-C, and HDL-C compared with policosanols, although they were not statistically significant, raising the question of whether pure VLCFA can lower cholesterol levels more efficiently than policosanols or its mixture with VLCFA. The present study was performed to examine the effect of pure VLCFA alone derived from montan wax at 2 doses in hamsters. No significant reductions in T-C and non-LDL-C were observed, whereas VLCFA at 50 mg/kg BW per day significantly increased plasma HDL-C levels.

As policosanols and VLCFA have very low fat solubility, the delivery form of VLCFA could exist as another factor influencing the efficiency of VLCFA on cholesterol metabolism. Previous experiments showed significant changes in cholesterol levels when policosanols or VLCFA were delivered within an acacia gum-water vehicle through gastric gavage [3-5,15,48]. In the present study, inclusion of LT in VLCFA was thought to increase the solubility of VLCFA and thus improve the efficacy of VLCFA on cholesterol metabolism. The results showed that the combination of VLCFA and LT did not alter non-HDL-C and TG levels as compared with VLCFA alone or control. Interestingly, hamsters given 25 mg/kg BW per day VLCFA and 1 g/kg BW per day LT produced higher HDL-C levels than controls and those fed 50 mg/kg BW per day VLCFA alone. As the effect of LT alone on lipid levels was not assessed in the current study, it is not possible to determine whether the increased HDL-C is due to the effect of VLCFA or LT or the combination of both. It is well established that increased HDL-C levels are beneficial to human health by reducing the risk for developing cardiovascular disease [49]. The elevated HDL-C levels by VLCFA, especially when combined with LT, may benefit the cardiovascular system. However, more investigations are needed to ascertain this effect.

Cholesterol biosynthesis as measured using deuterium incorporation and gas chromatograph–mass spectrometry [39,50] did not support previous work, which demonstrated an inhibition of policosanols on cholesterol biosynthesis using indirect methods [5,18,19]. The present study showed increases in cholesterol biosynthesis in hamsters after supplementation with VLCFA. However, the differences between the treatments for the estimated cholesterol synthesis rate were very small, suggesting that dietary treatment may have little physiological impact on cholesterol biosyn-

thesis. Similarly, a recent study showed no action of policosanols on cholesterol biosynthesis as measured by deuterium incorporation and isotope ratio mass spectrometry in the same animal model given similar diets [16]. Because an atherogenic diet was applied in the present study, the large amount of cholesterol load from the absorption of dietary source may have resulted in a great suppression of cholesterol biosynthesis. As such, cholesterol biosynthesis could not be suppressed further by VLCFA, resulting in the observed ineffectiveness on cholesterol levels in the plasma. It should be pointed out that in the current study an average deuterium enrichment of free cholesterol found in humans and animals situated in the west of Montreal was used as the baseline for estimating the amounts of newly synthesized cholesterol of each hamster; hence the differences in the estimated cholesterol synthesis rate between individual animals might be reduced.

The combination of PS and VLCFA reduced plasma T-C and non-HDL-C without changing HDL-C and TG levels. Decreases in T-C have been observed previously in hamsters [25,38,47] and rabbits [51] fed a similar atherogenic diet supplemented with the same level of PS mixture compared with the controls. It has been demonstrated that PS lower plasma cholesterol levels by inhibiting intestinal absorption and enhancing fecal excretion [25,47]. As such, a compensatory upregulation of cholesterol biosynthesis occurs after PS supplementation [25,26]. The insignificant effect of VLCFA and PS combination on cholesterol biosynthesis in the current study might also be due to the aforementioned great suppression of cholesterol biosynthesis by feeding hamsters the atherogenic diet.

In summary, VLCFA had no lipid-lowering effect in hamsters given an atherogenic diet. Matrixing of VLCFA with LT did not reduce, but instead increased, cholesterol levels. Phytosterols consistently showed a significant cholesterol-lowering action. No additive effect was observed for the combination of VLCFA and PS in reducing cholesterol levels, which might be due to the ineffectiveness of VLCFA. A novel finding from the current study is that plasma HDL-C levels were elevated by VLCFA and further by the mixture of VLCFA and LT. Insignificant changes in T-C/HDL-C ratio indicate that the increased T-C after VLCFA feeding was a result of the increased HDL-C. Results of the current study support the cholesterol-lowering action of plant sterols in hamsters. Although VLCFA did not reduce plasma cholesterol levels, the elevated HDL-C by VLCFA may be beneficial to the cardiovascular system.

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