Effects of dietary behenate and a caprenin-like fat on lipids in the hamster

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To study the effects of dietary behenic acid on cholesterol metabolism, we fed hamsters four synthetic, randomly arranged triglycerides that were formulated to allow the comparison of the effects of a single dietary fatty acid. The dietary triglycerides (including that from chow) fed to the linoleate group contained approximately 55% linoleic acid. The palmitate, oleate, and behenate groups received fats similar in composition to the linoleate fat, but approximately half of the linoleic acid of the triglyceride was replaced with either palmitic, oleic, or behenic acid. In addition to this long-chain behenate fat (BLCT), behenic acid was also fed as a triglyceride made from medium-chain fatty acids and behenic acid (BMCT). This BMCT fat was similar in composition to caprenin, a reduced-calorie alternative to cocoa butter. A group that received medium-chain triglycerides (MCT) was included for comparison with the BMCT group. The synthetic fats were all fed at a level of 15% by weight, along with 84.8% chow and 0.2% cholesterol. After 4 weeks the animals were sacrificed and LDL cholesterol levels were measured. Measurements are mmol/L, common superscripts indicate no difference, $P < 0.05$.

Chow, 0.78 ± 0.05; Linoleate, 4.73 ± 0.23†, Palmitate, 5.07 ± 0.31†; Oleate, 2.79 ± 0.10‡,§; BLCT, 2.53 ± 0.13‡; BMCT, 3.26 ± 0.28 ± §; MCT, 4.16 ± 0.34¶. Behenic acid in feces was measured after methylation and extraction with ether. Behenic acid absorption ranged from 19–29%. The presence of unhydrolyzed triglyceride in the feces of the BLCT group suggested that BLCT might alter the absorption of dietary cholesterol. In a second study, fecal cholesterol and its metabolites were measured after feeding BLCT. The fecal cholesterol was nearly three times that from a safflower oil-fed control group.*

Keywords: caprenin; behenic; octanoic; decanoic; fecal fat; cholesterol

Introduction

It is generally accepted that long-chain saturated fatty acids are the most hypercholesterolemic of dietary components, and the equations formulated by Keys[1] and later by Hegsted[2] directly related increases in plasma cholesterol to increases in dietary saturated fatty acids. Later work by Keys, however, suggested that stearic acid did not belong in the group of cholesterol-raising fatty acids.[3] More recently, Grundy and Bonanome demonstrated that stearic acid was not hypercholesterolemic, presumably resulting from conversion of stearic to oleic acid after absorption into the body.[4]

The studies of the effects of dietary saturated fatty acids on blood cholesterol levels have not extended the chain length beyond the 18 carbon atoms of stearic acid. Although these very long-chain fatty acids are currently dietary components in foods such as peanut oil, peanut butter, and hydrogenated fish oil, there is a paucity of information about their effects on blood lipids.

Caprenin, a triglyceride that is formulated from 50% behenic acid and a mixture of caprylic (C8:0) and capric (C10:0) acids has been recently introduced as an alternative to cocoa butter. The incomplete absorption of the behenate and the inefficient utilization of the medium-chain fatty acids results in a reduced caloric density, approximately 20.9 kJ/g versus 37.7 kJ/

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g, traditionally assumed for dietary fats.[5] A recent study of a fat with a composition similar to that of caprenin in a short-term clinical trial showed it to be equivalent to soybean oil and medium-chain triglycerides in its effect on total cholesterol.[6]

To investigate the effects of behenic acid on cholesterol metabolism, we fed hamsters triglycerides containing behenic acid or other dietary fatty acids and measured plasma lipoprotein cholesterol levels (study 1) and cholesterol excretion (study 2). Behenic acid was fed as a randomized triglyceride made from behenic acid and long-chain fatty acids (BLCT) or as a randomized triglyceride made from behenic acid and medium-chain fatty acids (BMCT). The fatty acid composition of BMCT is similar to that of caprenin. A group fed MCT was included for comparison with BMCT in study 1.

Study 1 compared the effects of behenic acid incorporated into a triglyceride with those of other fatty acids incorporated into similar triglycerides. This comparison was made by synthesizing a series of triglycerides that varied a single fatty acid constituent while keeping the remaining composition constant. The single dietary variable among groups was the fatty acid of interest, and behenic acid was directly compared with linoleic, oleic, and palmitic acids. The 4-week duration of study 1 followed the work of Spady and Dietschy,[7] who showed differences in the effects of dietary triglycerides in the hamster after 30 days.

Study 2 was undertaken to investigate the mechanism of the low-density lipoprotein (LDL) cholesterol reduction that was observed in the BLCT group in study 1. The possible inhibition of dietary cholesterol absorption by BLCT was suggested by study 1. The 2-week duration allowed a 10-day fecal collection to be made to measure sterol excretion.

We also validated a method for the measurement of behenic acid in feces to determine the absorbability of behenic acid. To complement the behenate absorption measurements, we determined the level of fatty acids in the carcass and liver.

Materials and methods

Materials

Diets comprised 84.8% chow (Wayne Lab Blox Teklad, Madison, WI USA), 0.2% cholesterol (Sigma, St. Louis, MO USA), and 15% test fat. The test fats included sunflower oil (Wesson, Fullerton, CA USA), medium-chain triglyceride oil (MCT, Mead Johnson, Evansville, IN USA), and synthetic triglyceride fats prepared in these laboratories (by D.K. Yang) by base-catalyzed rearrangement of varying ratios of triglycerides (safflower oil, Hollywood, Los Angeles, CA USA; completely hydrogenated palm oil, and completely hydrogenated soybean oil, Procter & Gamble, Cincinnati OH USA; and high oleate sunflower oil, SVO, Eastlake, OH USA) with appropriate compositions to obtain triglycerides enriched in specific fatty acids. The BMCT fat comprised 27% C8:0, 20% C10:0, 5% C20:0, and 41% C22:0. The composition of the dietary fats (based on extracted fat from the chow and fat mixture) is given in *Table 1*. The material termed "stigmastanol" (Sigma) comprised 55% stigmastanol and 45% ergostanol as measured by gas chromatographic (GC) analysis of their trimethyl silyl ether (TMS) derivatives (see below). Stigmasterol (Sigma) used in cholesterol measurements described below was greater than 95% pure by GC analysis.

The validation of the fecal extraction procedure utilized completely hydrogenated rapeseed oil (6.5% palmitic acid, 54.4% stearic acid, 5.8% arachidic acid, 28.8% behenic acid, 0.9% lignoceric acid), and calcium behenate. The calcium behenate was prepared from behenic acid (99.7% by GC of its methyl ester, Sigma). The sodium soap of behenic acid was prepared by stirring and heating (steam bath) 50 g of the acid in 1 L of methanol with dropwise addition of a 0.1 N NaOH to attain a molar equivalent. The flask was cooled, and the resulting precipitate filtered and dried by lyophilizing. A 5% (wt) aqueous suspension of the sodium soap was heated with a steam bath and stirred, and an aqueous solution of calcium chloride (0.08 M) was added until a 10% molar excess of calcium was present. The suspension was heated and stirred for 1 hr. After cooling, the resulting precipitate was filtered, washed with distilled water, and dried in air at room temperature. A portion was ashed with perchloric acid and analyzed for calcium by atomic absorption spectroscopy. Calcium found was 5.4% (wt., 5.6% theory).

Animals

Male golden Syrian hamsters (Charles River Laboratories, Wilmington, MA USA) weighing 62–95 g were used in these studies. In the first study, the animals were housed three per cage, and in the second, two per cage. The cages were fitted with wire screen floors to facilitate fecal collection. Food cups were refilled Monday, Wednesday, and Friday of each week. Diets and tap water were provided ad libitum. Food consumption was recorded with each filling of the food cups.

The length of the first study was 4 weeks. Two 7-day pooled fecal collections were made on days 6–20. The second study included 14 days of feeding with a pooled fecal collection on days 4–14. Animals were sacrificed by inhalation of carbon dioxide at the conclusion of each study. Tissues and blood samples were taken immediately after sacrifice.

Plasma and tissue samples

A sample of nonfasting plasma was separated into lipoprotein fractions according to the method of Havel et al.[8] The LDL cholesterol was measured in the 1.019–1.063 fraction by saponification (2 wt% KOH, 1 hour), extraction, GC analysis (DB-17 column, 0.25mm × 30m, 25 micron film thickness, flame ionization detector), and comparison with a known quantity of stigmasterol that was added to the plasma before saponification. Cholesterol levels in the $d < 1.019$ and $d > 1.063$ fractions were also determined by the same procedure. Plasma triglycerides were measured by glycerol-blanked, enzymatic method (Hitachi 717 clinical chemistry analyzer, Boehringer Mannheim, Indianapolis, IN USA). Livers were extracted with chloroform:methanol (2:1, vol/vol), and total cholesterol was assayed by GC as described above. Aliquots of these extracts were also saponified and converted to methyl esters[9] for GC analysis.

Carcasses were ground with dry ice in a Wiley mill, mixed with water, frozen, and lyophilized. Samples of the carcass were extracted with chloroform:methanol (2:1), and the fatty acid composition measured by GC after saponification and esterification as described above.

Table 1 Dietary fat compositions

Study 1—Comparison of six fats on plasma lipids

Fatty acid	Group						
	Chow	Linoleate	Palmitate	Oleate	BLCT	BMCT	MCT
8:0	—	—	—	—	—	16.8	50.3
10:0	—	—	—	—	—	12.7	21.8
12:0	—	—	—	—	—	0.2	0.3
14:0	1.5	0.4	0.8	0.4	0.4	0.4	0.4
16:0	14.9	9.9	33.6	8.7	6.4	3.7	4.3
16:1	2.0	0.5	0.5	0.5	0.4	0.5	0.6
18:0	3.8	20.0	24.5	18.6	22.1	2.5	1.1
18:1	20.6	11.8	7.7	36.2	6.9	4.6	5.8
18:2	45.3	54.8	30.5	32.8	29.9	10.5	13.3
18:3	4.5	1.0	1.0	1.1	0.9	1.0	1.3
20:0	0.4	0.4	0.5	0.4	4.5	4.8	—
20:5	1.7	0.4	0.4	0.4	0.4	0.4	0.6
22:0	0.3	0.3	0.1	0.5	26.8	40.8	0.2
22:6	1.6	0.3	0.3	0.2	0.3	0.4	0.4
24:0	—	—	—	—	0.8	0.7	—

Study 2—Cholesterol absorption study

Fatty acid	Group		
	Sunflower	Sunflower/Stigmastanol	BLCT
14:0	0.3	0.3	0.3
16:0	8.6	8.4	6.7
16:1	0.5	0.4	0.4
18:0	4.5	4.5	22.4
18:1	21.7	21.7	9.0
18:2	60.9	61.1	27.0
18:3	1.2	1.2	1.1
20:0	0.3	0.3	4.4
20:5	0.2	0.2	0.2
22:0	0.6	0.6	26.8
22:6	0.2	0.2	0.2
24:0	0.2	0.2	0.9

Diets comprised 84.8% rodent chow, 0.2% cholesterol, and 15% test fat. The chow included 5.6% total fat and 0.02% cholesterol. The compositions given are those of the total diet extracts.

Fecal analyses

Fecal samples were homogenized with water, frozen, and lyophilized. Samples that were used for fatty acid measurements were refluxed in methanol and sulfuric acid to convert all fecal fat to methyl esters. The samples were then extracted with ethyl ether, dried, weighed, and analyzed by GC. The validation of this methylation procedure is described in a separate section below.

Soxhlet extraction of a portion of the dried feces with chloroform and methanol (2:1) was used to obtain the unhydrolyzed fecal triglycerides. Unlike the insoluble high melting soaps, the triglycerides were readily solubilized in this process. Florisil column chromatography isolated the triglyceride from the extract.

Separate aliquots of the dried feces were used for cholesterol measurement in the second study. These samples were extracted with chloroform and methanol (2:1). An amount of 5-alpha cholestane standard was added, and the sample dried under a stream of nitrogen. Sterols were converted to the trimethyl silyl ether derivative (Sylon BTZ, Supelco, Bellefonte, PA USA), and then quantified by GC (30m x 0.25 mm i.d., RTX-5, 0.5 micron column). Coprostanone, and the TMS coprostanol and cholesterol derivatives were measured and their total mass used as the determinant of free (unesterified) cholesterol excretion.

Statistical comparisons

Comparisons among groups were made by analysis of variance and the calculation of the critical difference between groups based on the estimate of common variance of the treatment populations and two-tailed t test at $P < 0.05$.[10]

Validation of fecal behenate recovery

The recovery of behenic acid in the feces used a method that we had previously validated chemically and in rats. This method is an efficient means for the recovery of high-melting lipids and soaps from fecal matter, and the validation experiments are summarized below.

Calcium behenate was mixed in known proportions with lyophilized feces from rats that had been maintained with a chow diet (Purina, St. Louis, MO USA). The recovery of behenic acid was measured by four methods: (1) soxhlet extraction of 1.0 g with 2:1 (vol/vol) chloroform:methanol

for 18 hr; (2) soxhlet extraction of 1.0 g with ethyl ether for 18 hr; (3) direct saponification of 100 mg of the feces-soap mixture followed by methylation of the fatty acids with BF_3-methanol and quantifying by GC with a known added mass of pentadecanoic acid (Nu Chek Prep, Elysian, MN USA) added as a standard; and (4) methylation of 100 mg of the sample by refluxing in 2% (wt) sulfuric acid in methanol for 1 hr followed by extraction of the resulting methyl esters with 5 volumes of ethyl ether.

The results were obtained from triplicate samples (weight percent) and are shown in *Table 2*.

Based on the theoretical yields (32.8, 49.3, and 65.7% for the 2:1, 1:1, and 1:2 ratios, respectively), the methylation procedure provided essentially complete behenate recovery even at high levels of the insoluble divalent soap.

Feces were also obtained from rats that had eaten diets comprising 25% casein, 52% sucrose, 3% celluflour, 0.5% AIN 76 vitamin mix, 4% salt mix XVII, and 0.3% choline chloride and 15% completely hydrogenated high erucic rapeseed oil (CSP Foods, Winnipeg, MB, Canada total diet fat fatty acid composition: palmitic acid, 11%; stearic acid, 36%; arachidic acid, 4%; behenic acid, 22%; arachidic acid, 1%; oleic acid, 11%, linoleic acid, 15%). These lyophilized feces were extracted by all methods described above except the ether soxhlet extraction method. In addition to the three methods described above, samples were acidulated by refluxing in 1 N HCl for 1 hour and then extracted by 2:1 chloroform methanol (followed by washing the chloroform phase with phosphate buffer after standing overnight). As shown below, the methylation and the acidulation-chloroform method were approximately equivalent and more complete than the other methods ($n=3$, mean ± SEM). Fecal lipid as a percent of dried feces for the $CHCL_3$MeOH method was 58.3 ± 2.1; saponification, 61.1 ± 0.3; methylation, 70.6 ± 0.4; and HCl/$CHCl_3$:MeOH, 72.5 ± 0.3. The methylation-extraction procedure was found to be a rapid and efficient method for the determination of fecal fat containing high-melting fatty acid derivatives.

Table 2 Results of behenate fecal recovery

Ratio of Feces/soap	Method			
	$CHCl_3$-MeOH	Ether	Saponification	Methylation
2:1	19.3 ± 0.3	1.8 ± 0.1	27.7 ± 0.9	32.8 ± 0.6
1:1	17.8 ± 0.9	1.9 ± 0.5	35.0 ± 1.1	48.0 ± 1.2
1:2	16.9 ± 1.7	1.4 ± 0.4	46.7 ± 3.8	60.8 ± 1.6

Results

Study 1 (plasma and tissue cholesterol measurements)

Weight gain and food consumption. The body weights and food consumption observed for each group are shown in *Table 3*. The linoleate group gained less weight than the other groups that received fat added to the chow. Its diet consumption was consistent with the lower weight gain although not significantly lower than that of the other groups.

Plasma cholesterol. The plasma triglycerides and lipoprotein cholesterol concentrations are given in *Table 4*. The chow, oleate, and two behenate groups resulted in the lowest total and LDL cholesterol concentrations.

Liver Cholesterol. The liver cholesterol concentrations are also shown in *Table 4*. The cholesterol in the livers of the animals fed chow, BLCT, or BMCT were lower than those from the other groups, and that in the oleate group livers was the highest.

Tissue fatty acids. The fatty acids in the carcass and the liver are presented in *Table 5*. The level of behenic

Table 3 Weight gain (g) and food consumption (g), study 1

	Group						
	Chow	Linoleate	Palmitate	Oleate	BLCT	BMCT	MCT
Diet consumed	28 ± 0.6	24 ± 0.8	28 ± 1.4	27 ± 0.4	29 ± 1.6	30 ± 0.8	29 ± 2.2
Weight gain	48 ± 3.2^{a}	50 ± 4.1^{a}	57 ± $3.6^{a,b}$	62 ± 3.1^{b}	53 ± $4.8^{a,b}$	63 ± 3.1^{b}	58 ± $4.7^{a,b}$

Food consumption is average daily consumption/three animals (n = 3). Weight gain is total gain/animal (n = 9). Mean ± SEM. In this table and in subsequent tables, mean values within a row and having different superscripts differ ($P < 0.05$).

Table 4 Plasma lipoprotein cholesterols (mmol/L), liver cholesterol (mmol/kg of liver), liver weights (g/100 g body weight) from study 1

	Chow	Linoleate	Palmitate	Oleate	BLCT	BMCT	MCT
VLDL*	0.39 ± 0.05^{a}	8.97 ± 1.3^{b}	14.3 ± 1.5^{c}	4.09 ± 0.44^{d}	2.66 ± 0.28^{d}	1.42 ± 0.08^{d}	3.34 ± 0.59^{d}
LDL	0.78 ± 0.05^{a}	4.73 ± $0.23^{b,e}$	5.07 ± 0.31^{b}	2.79 ± $0.1^{c,d}$	2.53 ± 0.13^{c}	3.29 ± 0.28^{d}	4.16 ± 0.34^{e}
HDL	1.78 ± 0.10^{a}	2.59 ± 0.10^{b}	2.43 ± 0.13^{b}	3.18 ± 0.08^{c}	2.17 ± 0.10^{b}	2.46 ± 0.13^{b}	3.41 ± 0.18^{c}
Liver cholesterol	5 ± 0.3^{a}	70 ± 5^{b}	47 ± 3^{c}	93 ± 3^{d}	34 ± 3^{e}	16 ± 0.1^{a}	72 ± 3^{b}
Liver mass	4.6 ± 0.1^{a}	6.0 ± 0.3^{b}	5.8 ± $0.1^{b,c}$	6.0 ± $0.1^{b,c}$	5.4 ± 0.1^{b}	5.8 ± $0.1^{b,c}$	6.4 ± 0.3^{c}

*VLDL includes all d < 1.019 particles.
Mean values within a row and having different superscripts differ ($P < 0.05$).

Table 5 Tissue fatty acid composition (%) from study 1

Liver fatty acids							
	Group						
Fatty acid	Chow	Linoleate	Palmitate	Oleate	BLCT	BMCT	MCT
10:0	—	—	—	—	—	0.4	0.4
14:0	0.1	0.2	0.2	0.2	0.2	0.3	0.4
16:0	20.7	11.7	17.1	10.4	13.8	18.1	14.0
16:1	1.6	1.2	1.1	1.3	1.1	1.4	2.5
18:0	15.9	16.4	19.4	13.0	15.0	13.6	11.4
18:1	18.2	22.1	20.3	35.6	18.1	17.2	29.4
18:2	16.5	25.9	20.5	19.1	25.0	17.2	14.9
18:3	0.6	0.6	0.4	0.4	0.5	0.5	0.8
20:4	8.3	5.9	5.3	4.3	7.1	7.1	4.5
20:5	2.9	0.3	0.6	0.5	0.8	2.7	1.4
22:0	—	—	—	—	4.1	5.8	—
22:6	10.1	5.9	6.3	5.0	6.6	7.4	5.9
Carcass fatty acids							
	Group						
Fatty acid	Chow	Linoleate	Palmitate	Oleate	BLCT	BMCT	MCT
8:0	—	—	—	—	—	0.6	0.9
10:0	—	—	—	—	—	5.0	5.3
12:0	—	—	—	—	—	0.3	0.3
14:0	1.5	0.9	1.3	1.0	1.1	1.7	1.8
16:0	22.1	16.2	27.9	15.8	17.2	21.8	22.7
16:1	7.3	3.3	4.1	3.3	3.8	5.4	5.1
18:0	2.9	8.1	8.0	7.4	8.1	4.7	3.7
18:1	39.1	28.3	29.9	42.9	31.4	34.1	37.1
18:2	16.0	33.0	17.7	19.8	26.3	14.4	11.8
18:3	1.1	0.6	0.5	0.6	0.7	1.1	0.8
20:0	—	—	—	—	0.6	0.7	—
20:3	0.2	0.2	0.2	0.2	0.3	0.3	0.2
22:4	0.3	0.2	0.2	0.1	0.3	0.2	0.2
22:0	—	—	—	—	0.7	0.8	—
22:6	0.3	0.2	0.1	0.1	0.2	0.3	0.2
24:0	—	0.2	—	—	—	—	—

acid in the carcass can be calculated as mass of recovered behenic acid and compared with the total mass of behenic acid ingested during the study. The behenic acid in the carcass accounted for 1% of the behenic acid that was ingested during the 4 weeks of either the BLCT or the BMCT test diets.

Fecal fat. Table 6 displays the fatty acid recovery from the fecal collections obtained from the animals that received either BLCT or BMCT. Absorption of the long-chain fatty acids decreased with increasing chain length, and recovery of the behenic acid ranged from 70.9 to 80.9% of that ingested during the 7 days of fecal collection.

Compared with the long-chain saturated fatty acids, the excretion of the medium-chain and long-chain unsaturated fatty acids was very low. Fecal linoleic and oleic acids accounted for 1.5 and 0.5% of the fecal fatty acids, respectively, compared with levels of 29.9 and 6.9% of the dietary fatty acids, respectively, in the BLCT diet. Similarly, the fecal fat from the BMCT group contained less than 1% of octanoic acid, decanoic acid, linoleic acid, or oleic acid, although these acids were a significant part of the dietary fat *(Table 1.)*

Table 6 Fecal stearic (18:0), arachidic (20:0), behenic (22:0), and lignoceric (24:0) acids from study 1

	Group			
Period	18:0	20:0	22:0	24:0
BLCT, 1	56.0 ± 3.2	63.7 ± 7.6	80.9 ± 5.8	97.9 ± 6.7
BMCT, 1	48.4 ± 3.2	62.9 ± 5.3	75.4 ± 9.8	81.9 ± 4.7
BLCT, 2	48.4 ± 3.2	62.9 ± 5.3	75.4 ± 5.7	81.9 ± 4.7
BMCT, 2	46.0 ± 1.9	58.6 ± 0.7	70.9 ± 0.9	75.1 ± 1.9

Acids are shown as percentage of that ingested for the groups fed fats containing behenic acid (BLCT and BMCT groups) during two separate 1-week fecal collection periods (based on collections from each cage housing three animals, $n = 3$ cages).

The feces from the BLCT group contained a mass of unhydrolyzed triglyceride equivalent to 8.0 ± 0.5 (SEM)% of the dietary BLCT. The level of unhydrolyzed triglyceride in the BMCT group was markedly less, 1.1 ± 0.2% of the dietary BMCT.

Study 2 (cholesterol absorption)

This study compared the excretion of cholesterol and its principal metabolites from hamsters fed a diet with either safflower oil, BLCT, or safflower oil with stigmastanol. Stigmastanol is known to inhibit cholesterol absorption[11] and was included as a positive control.

Weight gain and food consumption. The weight gain and food consumption are shown in *Table 7*. The stigmastanol group ate less than the other two groups.

Plasma lipids. The plasma lipid values are given in Table 8. The total cholesterol of the safflower group was greater than that of the other two groups. The LDL cholesterol level of the stigmastanol group was less than that of the other two.

Cholesterol excretion. The excreted cholesterol (the sum of fecal cholesterol, coprostanol, and coprostanone) and the fraction that was not converted to coprostanol and coprostanone were calculated for each of the three groups. In terms of the percentage of cholesterol ingested during the 10-day fecal collection period, the sunflower oil-fed group excreted 27 ± 2.2%; the BLCT group, 75 ± 5.8%; and the stigmastanol group, 94 ± 5.0%.

Discussion

The data presented above show that triglycerides containing behenic acid significantly reduced plasma total and LDL cholesterol in the cholesterol-fed hamster when compared with the linoleate and palmitate-containing fats. Based on the observation of the effect of BLCT cholesterol excretion seen in the second study, it is possible that altered cholesterol absorption is at least partly responsible for the low LDL and very low density lipoprotein (VLDL) cholesterol levels resulting from that fat. This effect on cholesterol absorption is consistent with the presence of unhydrolyzed triglyceride in the feces of the animals fed BLCT in the first study. Unhydrolyzed dietary fat has been shown to inhibit cholesterol absorption when compared with hydrolyzable triglycerides.[12]

In contrast, the BMCT group did not excrete abnormal quantities of triglyceride. This result would be predicted from the study of the lipase-catalyzed hydrolysis of caprenin.[13] Caprenin, which is similar in composition to the BMCT fat, was shown to hydrolyze in a manner that was identical to typical triglycerides. Given the absence of the unhydrolyzed fat in the feces, it is unlikely that caprenin would affect cholesterol absorption.

Table 7 Weight gain and food consumption from study 2

	Group		
	Sunflower	Sunflower/ Stigmastanol	BLCT
Diet consumed g/animal/day	8.0 ± 0.9[a,b]	7.2 ± 0.5[a]	8.7 ± 1.2[b]
Weight gain g/animal/14 days	22.8 ± 2.7[a]	14.2 ± 2.8[b]	15.7 ± 1.4[b]

Mean values within a row and having different superscripts differ ($P < 0.05$).

Table 8 Plasma lipoprotein cholesterols (mmol/L) from study 2

	Group		
	Sunflower	Sunflower/stigmastanol	BLCT
VLDL	1.97 ± 0.09[1]	0.28 ± 0.05[2]	0.08 ± 0.10[3]
HDL	3.25 ± 0.07[1]	2.34 ± 0.09[2]	2.42 ± 0.07[2]
LDL	0.92 ± 0.07[1]	0.21 ± 0.05[2]	0.74 ± 0.10[3]

Mean values within a row and having different superscripts differ ($P < 0.05$).

The calculation of the fractional absorption of dietary behenic acid from its fecal recovery should not be affected by potential loss of the acid due to microflora during gastrointestinal transit. The metabolism of fatty acids in the large intestine is limited to anaerobic reactions that hydroxylate and hydrogenate unsaturated acids but do not alter long-chain saturated acids.[14]

The fatty acids of BMCT and BLCT did not contribute to blood cholesterol elevation. Behenic acid from dietary BMCT and BLCT was poorly absorbed and accumulated only in trace amounts in the carcass and liver. There was no evidence of its conversion to fatty acids associated with hypercholesterolemia such as palmitic and myristic acids. In addition, the octanoic and decanoic acids, although well absorbed, are not associated with elevations of blood cholesterol levels.[15]

The high level of cholesterol in the livers of the high oleic acid-fed animals seen in this study has been observed by others.[16] The low LDL cholesterol level seen in this group is consistent with Dietschy's observation of the absence of a correlation of total liver cholesterol with LDL receptor activity.[17]

The hamster was chosen for these studies because its total and LDL cholesterol levels have been shown to change with alterations in dietary fat in a manner similar to the changes in humans. Studies of LDL metabolism in the hamster have shown increases in LDL with the feeding of saturated fat and cholesterol resulting from reductions in LDL receptor activity and increases in LDL production.[7] Also in the hamster, MCT has been shown to reduce LDL cholesterol, enhance receptor-dependent LDL transport, and lessen fat-induced LDL production compared with more typical saturated fatty acid triglycerides.[15] The results that we report here are generally consistent with these published observations. The LDL cholesterol observed in the linoleate group was somewhat higher than might be predicted, but the oleate and MCT groups were consistent with the previously reported data. The fatty acids of the BLCT and BMCT groups did not contribute to elevated LDL cholesterol, and LDL cholesterol was significantly reduced in these groups compared with the group fed the palmitate fat.

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