Behenic acid is a cholesterol-raising saturated fatty acid in humans[1–3]

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ABSTRACT

Background: Dietary behenic acid (22:0) is poorly absorbed. Because of its low bioavailability compared with other fatty acids and because of its very long chain length, the effect of dietary behenic acid (behenate) on serum lipid concentrations in humans is assumed to be neutral.

Objective: The objective was to establish the cholesterol-raising potential of behenic acid by comparing the effects on lipid and lipoprotein concentrations of a specially formulated fat enriched with behenic acid with those of palm oil (rich in palmitic acid; 16:0) and high–oleic acid sunflower oil (rich in *cis* oleic acid; 18:1).

Design: In a randomized, crossover, metabolic-ward study, 7 mildly hypercholesterolemic men were fed 3 natural-food diets supplemented with behenate oil, palm oil, or high–oleic acid sunflower oil. Mean serum lipid and lipoprotein concentrations and plasma triacylglycerol fatty acid composition were determined from fasting blood drawn during the final 4 d of each 3-wk diet period.

Results: Behenate oil produced mean concentrations of total cholesterol (5.87 ± 0.8 mmol/L) and LDL cholesterol (4.40 ± 0.8 mmol/L) not significantly different from those produced by palm oil (5.84 ± 0.7 and 4.42 ± 0.7 mmol/L, respectively) but significantly higher than those produced by high–oleic acid sunflower oil (5.12 ± 0.5 and 3.70 ± 0.6 mmol/L, respectively). There were no significant differences in triacylglycerol or HDL-cholesterol concentrations.

Conclusions: Despite its low bioavailability compared with oleic acid, behenic acid is a cholesterol-raising fatty acid in humans and is therefore not a suitable substitute for palmitic acid in manufactured triacylglycerols. *Am J Clin Nutr* 2001;73:41–4.

KEY WORDS Behenic acid, saturated fatty acids, dietary fat, cholesterol, lipids, carbon chain length, men

INTRODUCTION

The carbon chain length of a saturated fatty acid has long been suspected to be an important determinant of its cholesterol-raising potential. The importance of fatty acid saturation was noted in early equations developed by Keys et al (1) and Hegsted et al (2). Both investigators estimated the relative contribution of fatty acid type to the cholesterol-raising potential of a fat on the basis of data collected by using naturally occurring fats that had a predetermined fatty acid mixture. Specifically, the superiority of polyunsaturated fatty acids was likely overestimated by Keys et al (1) and Hegsted et al (2) because polyunsaturated fatty acids predominated only in vegetable oils that were also low in saturates. Monounsaturated fatty acids, on the other hand, were found in fats both high and low in saturated fatty acids and appeared more neutral. Examination of the role of fatty acid chain length was also limited by technology because >90% of naturally occurring saturated fatty acids have chain lengths of 12–18 carbons (3).

The cholesterol-raising potential of specific saturated fatty acids has received renewed interest because advances in biotechnology have permitted the formulation of dietary fats containing specific fatty acids. These advances in biotechnology permit more widespread consumption of less common fatty acids, the clinical effects of which have not been characterized. We embarked on a systematic evaluation of whether chain length per se was the critical factor determining the cholesterol-raising potential of saturated fatty acids. The inability of stearic acid (18:0) to raise serum total and LDL-cholesterol concentrations was confirmed in our center by evaluating a manufactured fat enriched with stearic acid (4) and by carefully comparing fats with different natural enrichments of stearic acid (5). We reported recently that medium-chain saturated fatty acids [caprylic acid (8:0) and capric acid (10:0)] are in fact cholesterol raising, with approximately one-half of the potency of palmitic acid (16:0) (6). Lauric acid (12:0) appears to have two-thirds of the cholesterol-raising potential of palmitic acid (7). Myristic acid (14:0) appears to have even greater cholesterol-raising potential than does palmitic acid (8).

In the present study, we evaluated the cholesterol-raising potential of behenic acid (22:0). Behenic acid is poorly absorbed; the absorption rate of palmitic acid is ≈95–98% (9) and that of behenic acid is ≈30% (10). Because of its very long chain length and its low bioavailability compared with other fatty acids, it has been speculated that behenic acid does not significantly affect cholesterol concentrations (11). To determine whether behenic acid has cholesterol-raising potential, we compared the effects on lipid and lipoprotein concentrations of a specially formulated fat enriched with behenic acid with those of

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[2] Supported by NIH Endocrinology and Metabolism training grant 5 T32 OK07307 (to NBC), NIH-NIHLBI Clinical Investigator Award NIH5-K08-HL02456 (to MAD), and grant NIH R37HL29252-15.

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Received February 1, 2000.

Accepted for publication May 22, 2000.

palm oil (rich in palmitic acid) and high–oleic acid sunflower oil (rich in *cis* oleic acid; 18:1). If behenate has cholesterol-raising properties similar to those of palmitate, behenic acid would have only one-third of the cholesterol-raising potential of palmitic acid. If the cholesterol-raising properties of behenate are more similar to those of stearic acid, no differences in lipid concentrations between the high–oleic acid sunflower oil and the behenate oil should be observed.

SUBJECTS AND METHODS

Subjects

Seven men with mild hypercholesterolemia followed by the investigators in their lipid clinic were recruited to participate in this study. These men were aged 55–75 y ($\bar{x}$: 66 y) and had a mean body mass index (in kg/m^2) of 27 ± 5. At baseline, the mean (±SD) fasting concentration of total cholesterol was 5.69 ± 0.54 mmol/L (219 ± 21 mg/dL), that of triacylglycerol was 1.52 ± 0.77 mmol/L (135 ± 68 mg/dL), that of LDL cholesterol was 4.0 ± 0.60 mmol/L (155 ± 24 mg/dL), and that of HDL cholesterol was 0.93 ± 0.10 mmol/L (36 ± 5 mg/dL). Three men had documented coronary heart disease but were asymptomatic at the time of the study. All subjects were medically stable and had no evidence of renal disease, gastrointestinal disease, untreated endocrine disease, or glucose intolerance, defined as a fasting glucose concentration >5.5 mmol/L (>100 mg/dL) or a random glucose concentration >7.2 mmol/L (>130 mg/dL). Informed consent was obtained from each patient and the research protocol was approved by the Subcommittee of Human Studies of the Dallas Veterans' Administration Medical Center.

Study design

We used a randomized, single-blind, crossover design for this metabolic-ward study. There were 3 diet periods, each lasting 3 wk. Patients lived in the metabolic ward during each dietary period and were provided with all foods and fat supplements. Each dietary period was separated by ≥1 wk of an outpatient, ad libitum dietary period. During each of the final 4 d of each dietary period, blood was drawn after a 14-h fast. Mean lipid and lipoprotein concentrations from the final 4 d of each period were used as indicators of the subjects' responses to the diets.

Energy composition of diets

Each of the 3 diets consisted of low-fat, natural foods and 1 of 3 fat supplements. The overall energy composition of the diet was 53% fat, 35% carbohydrate, and 12% protein. The base diet contributed the recommended amount of daily energy from carbohydrate (35%) and protein (12%) and 10% of the total required amount of fat (3% saturated, 4% monounsaturated, and 3% polyunsaturated fatty acids); dietary cholesterol averaged 91 mg/d. Daily energy from fat in the base diet came from lean meats, skim milk, and margarine.

Fatty acid composition of fat supplements

The daily fat supplements contributed 43% of daily energy. Fat supplements provided to patients during each of the 3 dietary periods consisted of *1*) palm oil (RBD Palm Oil; Anderson-Clayton, Memphis), *2*) high–oleic acid sunflower oil (Trisun 90 High Oleic Sunflower Oil; SVO Enterprises, Eastlake, OH), or *3*) behenate oil (Proctor & Gamble, Cincinnati).

TABLE 1
Fatty acid composition of the fat supplements[1]

	Palm oil	High–oleic acid sunflower oil	Behenate oil
		% by wt	
14:0	1.1	ND	1.2
14:1	ND	ND	0.8
16:0	48.3	3.5	3.2
16:1	0.2	0.1	ND
18:0	4.4	3.3	3.9
18:1	35.0	87.0	45.5
18:2	7.6	5.0	2.2
18:3	ND	ND	1.9
22:0	ND	ND	39.5

[1] Composition determined by gas chromatography. ND, not detected.

The fatty acid composition of the 3 fat supplements is shown in **Table 1**. The concentration of behenic acid in the behenate oil was similar to the concentration of palmitic acid in palm oil. Anticipating that only 30% of behenate would be absorbed, we increased the daily allotment of behenate oil by 24% so that the same amount of absorbable energy from test fat was provided in all dietary periods.

Patients were instructed to add the fat supplements to soups, cereals, breads, and vegetables under supervision by the metabolic-ward staff. Each patient was interviewed daily and trays were examined to ensure that all food was consumed and that no oil remained on the plate. Total daily energy intake was adjusted as necessary for each patient to maintain a constant weight throughout the study. Patients were allowed to walk around the hospital grounds but were not allowed to engage in strenuous physical activity.

Lipid and lipoprotein analyses

Plasma concentrations of cholesterol and triacyglycerol were determined by enzymatic assay (12, 13). Apolipoprotein B–containing lipoproteins were precipitated by heparin manganese, after which plasma HDL-cholesterol concentrations were determined (14). VLDL (density < 1.006 kg/L) was isolated by ultracentrifugation (1.5×10^5 *g*, 18 h, 12–16°C) and the cholesterol concentration in the VLDL fraction and the infranate was measured. The LDL-cholesterol concentration was determined by calculating the difference between the infranate cholesterol and the HDL fraction. Results were adjusted according to the percentage recovery based on the differences between total cholesterol and the VLDL plus infranate cholesterol.

Plasma fatty acid analysis

The specific fatty acid composition of plasma triacylglycerol in each sample was determined by using the method of Lepage and Roy (15). Plasma lipids were extracted and triacylglycerols were separated on silica-gel G plates (250-μm thick) by thin-layer chromatography with hexane dietyl:ether:acetic acid (80:19:1, by vol) as the solvent. Triacylglycerol bands were scraped into glass tubes and resuspended in 2-mL volumes of a solution of methanol:benzene (4:1). After 200 μL acetyl chloride was added, fatty acids were transesterified by heating the samples at 100°C for 1 h. The fatty acid composition was determined by gas-liquid chromatography (Hewlett-Packard, Palo Alto, CA) on a capillary column.

TABLE 2
Plasma triacylglycerol fatty acid composition[1]

Fatty acid	Palm oil	High–oleic acid sunflower oil	Behenate oil
		% by wt	
14:0	1.1 ± 0.4[a]	1.0 ± 0[a]	1.6 ± 0.5[a]
16:0	28.1 ± 1.5[b]	20.7 ± 2.3[a]	23.7 ± 2.3[a]
18:0	3.9 ± 0.9[a]	3.1 ± 0.4[b]	4.1 ± 0.7[a]
22:0	ND	ND	1.0 ± 1.0
Total saturates	33.1 ± 2.3[b]	24.8 ± 2.5[a]	30.4 ± 3.3[b]
16:1	3.0 ± 1.2[a]	2.9 ± 1.5[a]	3.4 ± 1.1[a]
18:1	45.4 ± 2.4[b]	55.4 ± 6.3[a]	48.3 ± 2.1[b]
18:2	16.4 ± 2.1[a]	16.0 ± 2.8[a]	16.3 ± 2.1[a]
20:4	1.3 ± 0.8[a]	1.1 ± 0.4[a]	1.3 ± 0.5[a]
Total unsaturates	66.1 ± 3.1[b]	75.6 ± 2.3[a]	69.3 ± 3.0[b]

[1] $\bar{x}$ ± SD. Because of rounding, numbers may not add up to 100%. ND, not detected. Means within rows with different superscript letters are significantly different, $P < 0.01$.

Statistical analysis

The mean values obtained for the 3 dietary periods were compared by performing a repeated-measures analysis of variance (ANOVA). When the ANOVA showed the results of diets to be different, paired *t* tests with Bonferroni correction for multiple comparisons were performed (16). After Bonferroni correction, statistical significance was set at a *P* value of 0.0167 (<0.05/3).

RESULTS

The subjects tolerated the diets well and consumed all foods provided. There were no significant complaints of gastrointestinal discomfort related to the large intake of test oils.

The plasma triacylglycerol fatty acid composition during the 3 diets is shown in **Table 2**. Although the plasma triacylglycerol fatty acid composition during the palm oil and high–oleic acid sunflower oil diets reflected dietary fatty acid intakes, the composition of plasma triacylglycerol fatty acids during the behenate oil diet did not reflect dietary intakes. Only a small amount of behenic acid was detected in plasma during the behenate oil diet. The total amount of plasma saturated fatty acids during the behenate oil diet was significantly greater than that during the high–oleic acid sunflower oil diet but was not significantly different from the plasma saturated fatty acid content during the palm oil diet. Palmitic acid accounted for most of the plasma saturated fatty acids measured during the behenate oil diet. This amount was greater than that found during the high–oleic acid sunflower oil diet but less than that during the palm oil diet. Plasma triacylglycerol stearic and myristic acid concentrations were higher during the behenate oil diet than during the other 2 diets.

The plasma triacylglycerol unsaturated fatty acid content during the behenate oil diet was not significantly different from that during the palm oil diet. The plasma triacylglycerol unsaturated fatty acid content during the behenate oil and palm oil diets was significantly lower than that during the high–oleic acid sunflower oil diet. There tended to be a greater plasma triacylgycerol palmitoleic acid (16:1) content during the behenate oil diet than during the 2 other diets, although the differences were not significant.

Mean lipid and lipoprotein concentrations during each diet period are presented in **Table 3**. The total cholesterol concentration of 5.87 mmol/L (227 mg/dL) during the behenate oil diet was not significantly different from the total cholesterol concentration of 5.84 mmo/L (226 mg/dL) during the palm oil diet. Likewise, the LDL-cholesterol concentration of 4.40 mmol/L (170 mg/dL) during the behenate oil diet was not significantly different from the LDL-cholesterol concentration of 4.42 mmol/L (171 mg/dL) during the palm oil diet. These concentrations of total and LDL cholesterol during the behenate- and palm oil diets were significantly higher than those obtained during the high–oleic acid sunflower oil diet. The concentrations of triacylglycerols, VLDL cholesterol, and HDL cholesterol did not differ significantly between the 3 diets.

DISCUSSION

The finding that feeding fat specially formulated to be rich in behenic acid resulted in cholesterol concentrations that were not significantly different from those produced by palm oil feeding was surprising and the certainty of this finding must be questioned. The primary strength of our study was that the diets were fed in a metabolic ward under randomized crossover conditions. The fats used contained 83–90% of fatty acids from behenic acid, palmitic acid, or oleic acid, which permitted a good comparison of the effect of these fatty acids on serum cholesterol concentrations. We used very-high-fat diets to ensure that we could determine sufficiently whether behenic acid absorbed from the diet has cholesterol-raising potential. Each diet period lasted 3 wk, ensuring that serum lipid and lipoprotein concentrations had reached steady states.

There were several limitations to our study. First, we studied only a small number of subjects; however, the magnitude of the differences in cholesterol concentrations between the behenate oil and high–oleic acid sunflower oil diet periods allowed for meaningful comparisons. It is doubtful that different results would have been observed with a larger number of subjects. Second, we did not measure behenic acid absorption directly and did not attempt to track behenate metabolism with a tracer. Thus, we can only speculate about the mechanisms underlying our findings. Third, we used only a single-formulated fat containing

TABLE 3
Lipid and lipoprotein concentrations during each dietary period[1]

Lipid	Palm oil	High–oleic acid sunflower oil	Behenate oil
Total cholesterol			
(mmol/L)	5.84 ± 0.7[b]	5.12 ± 0.5[a]	5.87 ± 0.8[b]
(mg/dL)	226 ± 28	198 ± 21	227 ± 30
VLDL cholesterol			
(mmol/L)	0.52 ± 0.2[a]	0.54 ± 0.3[a]	0.65 ± 0.4[a]
(mg/dL)	20 ± 8	21 ± 10	25 ± 17
LDL cholesterol			
(mmol/L)	4.42 ± 0.7[b]	3.70 ± 0.6[a]	4.40 ± 0.8[b]
(mg/dL)	171 ± 28	143 ± 23	170 ± 30
HDL cholesterol			
(mmol/L)	0.88 ± 0.2[a]	0.91 ± 0.2[a]	0.88 ± 0.2[a]
(mg/dL)	34 ± 6	35 ± 6	34 ± 8
Triacylglycerol			
(mmol/L)	1.39 ± 0.5[a]	1.35 ± 0.6[a]	1.50 ± 1.0[a]
(mg/dL)	123 ± 42	120 ± 50	133 ± 87

[1] $\bar{x}$ ± SD. Means within rows with different superscript letters are significantly different, $P < 0.01$.

behenic acid. Whether similar findings would have been observed with a different mixture of fatty acids is unknown. Nonetheless, data from the present study and those from a study by Wardlaw et al (17) with a manufactured triacylglycerol composed almost entirely of behenic acid and medium-chain fatty acids indicate that behenic acid has cholesterol-raising properties. In that study, consumption of the manufactured triacylglycerol resulted in cholesterol concentrations not significantly different from those produced by butter or palm kernel oil.

Because of its carbon chain length (22:0) and its well-documented poor absorption, it is thought unlikely that behenic acid would affect cholesterol concentrations. The results from a study of the appearance of behenic acid in lymph samples from rats suggest that only 11–24% of dietary behenic acid is absorbed (18). The results from a study of the fecal content of behenic acid in hamsters suggests that only 19–29% of behenic acid is absorbed (19). In humans, who are known to have a greater capacity for absorbing stearic acid than do animals, fecal recovery of behenic acid suggests that the mean absorption of behenic acid is ≈30% (20).

Although we did not assess behenic acid absorption directly, the appearance of behenic acid in plasma triacylglycerol fatty acids (Table 2) as a rough measure of absorption suggests that little if any behenic acid was absorbed and distributed intact to the fatty acid pool. If 30% of dietary behenic acid was absorbed, how was it processed? The finding that behenate oil feeding resulted in high concentrations of plasma triacylglycerol myristic, palmitic, and stearic acids suggests that behenic acid may be hydrolyzed shortly after absorption into shorter-chain saturated fatty acids. In support of this suggestion are the results of a study by Bernhard and Vischer (21), who determined that absorption was ≈40% in rats fed deuterium-labeled behenic acid. The label was absorbed mainly in shorter-chain saturated fatty acids, particularly stearic, palmitic, myristic, and lauric acids. These findings suggest that dietary behenic acid is extensively degraded to cholesterol-raising saturated fatty acids. Whether behenic acid undergoes complete degradation to acetyl-CoA (2:0), which is then incorporated into the synthesis of de novo saturated fatty acids, or whether behenic acid undergoes specific site cleavage into more common saturated fatty acids, is unknown.

Although the mechanism requires further elucidation, our findings indicate that dietary behenic acid is particularly potent in raising total and LDL-cholesterol concentrations. We found that behenate oil feeding resulted in total and LDL-cholesterol concentrations that were not significantly different from those after palm oil feeding. However, if only 30–40% of the behenic acid we fed was absorbed, our results suggest that the cholesterol-raising potency of behenic acid is 2–3 times that of palmitic acid, which has an absorption rate of >95% (9). These findings add to the body of evidence that cholesterol-raising saturated fatty acids have different potencies (11). We showed previously that lauric acid is two-thirds less potent and that the medium-chain fatty acids (8:0 and 10:0) are one-half less potent than is palmitic acid in raising cholesterol (6, 7).

In summary, the results of this metabolic-ward investigation indicate that behenic acid raises cholesterol in humans, and to a greater extent than does palmitic acid. The findings of the present study and those of another study of ours with medium-chain triacylglycerol oil (6) suggest that behenic acid and medium-chain fatty acids are not suitable substitutes for palmitic acid in manufactured triacylglycerols.

We thank Anh Nguyen for her technical support and assistance, Cheryl Davis for her assistance with the diets, and Kathleen Gray, Regina Strowd, and the staff of the Metabolic Unit of the Dallas Veterans' Affairs Medical Center for their assistance with patient care.

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