Impact of Lemongrass Oil, an Essential Oil, on Serum Cholesterol

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To test the hypothesis that non-sterol mevalonate pathway end products lower serum cholesterol levels, we asked 22 hypercholesterolemic subjects (315 ± 9 mg cholesterol/dl) to take a daily capsule containing 140 mg of lemongrass oil, an essential oil rich in geraniol and citral. The paired difference in serum cholesterol levels of subjects completing the 90-day study approached significance ($P < 0.06$, 2-tailed t-test). The subjects segregated into two groups, one consisting of 14 subjects resistant to the protocol and the other consisting of 8 subjects who responded. Paired differences in cholesterol level at 30, 60 and 90 d for resistant subjects were +2 ± 6, +2 ± 7 and −1 ± 6 mg/dl; paired differences for the responding subjects were −25 ± 10 ($p < 0.05$), −33 ± 8 ($p < 0.01$) and −38 ± 10 ($p < 0.025$), respectively. The paired difference (+8 ± 4) in the cholesterol levels of six responders 90 days after the discontinuation of lemongrass oil was not significant.
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The cardio-protective action of the vegetarian diet in lowering serum cholesterol may be due in part to a variety of non-sterol mevalonate pathway end products (1) which act in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) at the non-sterol site of control (2). We found that dietary supplements (50 ppm) of geraniol, the first end product of the branched pathways of plant mevalonate metabolism, and citral, a mixture of the acyclic *cis* and *trans* prenylaldehydes geranial and neral, suppressed avian hepatic mevalonate synthesis (3). These monoterpenes are the major (>80%) constituents of lemongrass oil, a GRAS substance (Generally Regarded as Safe, Flavoring and Extracts Manufacturer's Association 2624) approved for use as a food additive (4,5,6). In this report we present evidence that the constituents of lemongrass oil effectively lowered the cholesterol levels of a subset of hypercholesterolemic human subjects.

METHODS

The putative cholesterol-lowering action of non-sterol end products of the mevalonate pathway was tested by administering capsules containing 140 mg lemongrass oil to hypercholesterolemic subjects recruited from the clientele of the Cardiac Rehabilitation Clinic, University of Wisconsin Hospitals and Clinics. Males who had undergone coronary artery bypass surgery within the past seven years and who maintained an elevated serum cholesterol level while adhering to a diet with strict limitations on fat, energy and cholesterol intakes were invited to participate in the study. Of the 26 patients who volunteered for the study, 22 qualified with a cholesterol level in excess of 250 mg/dl. The volunteers were interviewed by a clinical dietitian (GU) who recorded each subject's medication (β-blockers, calcium channel blockers, aspirin, thiazide diuretics) and dietary history. The dietitian also gave instructions for recording food intake during the three days preceding clinic visits. These visits were scheduled in January and at four week intervals for three months. Four-week supplies of gelatin capsules filled with 140 mg lemongrass oil containing isoprenoid end products (monoterpenoid aldehydes and alcohols), the equivalent of 350 mg cholesterol on a molar basis, were distributed at the initial and two succeeding visits. Fasting blood and dietary records were collected and the dietitian graded the protocol adherence of the volunteers using a scale of 1 (low) to 3 (high). Dietary intakes of energy, cholesterol, total fat, and classes of fatty acids were analyzed using Nutritionist III software (N-Squared Computing, Silverton, OR). The dietitian also reviewed the subject's overall pattern of his response to the cholesterol-lowering action of lemongrass oil. The blood was processed for the analysis of serum lipids. Cholesterol and triglyceride levels were determined using Sigma Diagnostic Kits (No. 351, Cholesterol, total and HDL and No. 338, Triglycerides, Sigma Chemical Company, St. Louis, MO). Low density and very low density lipoproteins were precipitated from the serum (100 μl) with 10 μl each of 9.7 mM phosphotungstic acid + 0.4 M $MgCl_2$. After standing for 5 min at room temperature, the mixtures were centrifuged at 2000 × *g* for 10 min, the supernatants removed and the levels of cholesterol in HDL determined. Sera from normocholesterolemic laboratory workers were interspersed with sera from the experimental subjects for analysis. The mean ± SE for the cholesterol level of the reference volunteers was 201 ± 3 mg/dl. Across the study the SD for the analysis of the 200 mg cholesterol standard was 2.3% of the mean. Triglyceride concentrations were determined in sera samples that had been held at −80°C for 9 months. Statistical evaluation employed the paired Student t-test with the subject's baseline value serving as the base for comparison. Means ± SEM are presented in the text. Lemongrass oil was donated by Aroma Resources Division of Biddle Sawyer Corporation, Keyport, NJ (refractive index @ 25°C, 1.483-1.489), and Bell Flavors and Fragrances, Inc., Northbrook, IL (refractive index @ 25°C, 1.481-1.491). The major constituents of lemongrass oil, quantitated by gas liquid chromatography, included geranial (42%), neral (32%), geraniol (4%), 3% each of myrcene and citronellal and 1-2% each of limonene, linalool and dipentene. The study was approved by the U.S. Center for Health Sciences Human Subjects Committee.

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Abbreviations: GRAS, Generally Regarded As Safe; HDL, high density lipoprotein; HMGR, 3-hydroxy-3-methylglutaryl reductase.

TABLE 1

Summary of Responses to Lemongrass Oil

	n	Paired Difference	t	P
a. Summary of paired t-tests				
30 days				
Combined	21	−7 ± 6[a]	1.313	NS
Resisters	14	+2 ± 6	0.254	NS
Responders	7[b]	−25 ± 10	2.667	0.05
60 days				
Combined	22	−8 ± 6	1.443	NS
Resisters	14	+2 ± 7	0.189	NS
Responders	8	−33 ± 8	3.890	0.01
90 days				
Combined	16[c]	−15 ± 7	2.094[e]	0.06[e]
Resisters	10	−1 ± 6	0.124	NS
Responders	6[d]	−38 ± 10	3.814	0.025

b. Responding subjects who returned for post-test evaluation

	n	Cholesterol (mg/dl)	Paired differences mg	P
Baseline	6	320 ± 17[a]		
30-day	6	290 ± 20	−30 ± 4	<0.025
60-day	6	296 ± 21	−24 ± 3	<0.025
90-day	5[d]	281 ± 15	−34 ± 4	<0.025
Post test	6	328 ± 15	+8 ± 4	NS

[a] SEM.

[b] A subject (responder) was vacationing.

[c] Five subjects (one responder) withdrew following 60-day visit.

[d] One subject (responder) was on vacation.

[e] t = 2.131 for $p < 0.05$, two-tailed.

TABLE 2

Baseline Lipids, Physical Characteristics and Dietary Intakes of Subjects Grouped as Responders (n = 8) and Resisters (n = 14)

	Responders	Resisters
Physical characteristics		
Age, yr	58.6 ± 2.7[a]	56.3 ± 0.4
Weight, kg initial	91.7 ± 5.1	82.9 ± 2.9
Weight, kg final	91.8 ± 4.5	83.2 ± 3.0
BMI, wt/ht	29.9 ± 1.8[b]	26.1 ± 0.9[b]
Baseline lipids		
Triglycerides, mg/dl	445 ± 94[b]	266 ± 75[b]
Cholesterol, mg/dl	318 ± 15	313 ± 12
HDL cholesterol, mg/dl	30.7 ± 3.9[c]	33.0 ± 1.7[d]
Dietary intake		
Energy, kcal	1807 ± 123	2080 ± 217
Cholesterol, mg/1000 kcal	112 ± 14	127 ± 13
SFA, energy %	14.9 ± 1.1	14.2 ± 1.2
MUFA, energy %	12.5 ± 1.0	14.1 ± 1.1
PUFA, energy %	8.5 ± 0.6	7.6 ± 0.8
P/S ratio	0.60 ± 0.12	0.65 ± 0.20

[a] SEM.

[b] Different values, $p < 0.05$.

[c] One subject's HDL cholesterol, 55.6 mg/dl, fell in the normal range.

[d] Two subjects' HDL cholesterol, 43.5 and 43.6 mg/dl, fell in the normal range.

RESULTS

The mean cholesterol level of the 16 hypercholesterolemic subjects who completed this 90-day test of the cholesterol-lowering efficacy of lemongrass oil decreased from 310 ± 11 to 294 ± 11 mg/dl. The paired t-test suggested a modest treatment effect ($p < 0.06$). Baseline and 90-day HDL cholesterol levels for the subjects were 32.2 ± 2.3 and 32.1 ± 1.7 mg/dl. On reviewing the data, we noted a subject-to-subject variability in response to the treatment, a variability in response which has been documented for normocholesterolemic subjects in other studies of dietary inputs which influence cholesterol levels (7). Overall, we identified eight subjects who consistently responded to the treatment; the remaining 14 were resistant. A summary of the paired t-tests of the responses of these subsets of subjects and of the combined subsets is given on Table 1a. The serum cholesterol levels of responders at 30, 60 and 90 days were 91.8 ($n = 7$, $p < 0.05$), 89.6 ($n = 8$, $p < 0.01$) and 87.8 ($n = 6$, $p < 0.025$) percent, respectively, of their baseline values.

To provide some confirmation that the fall in cholesterol was a response to the treatment, we obtained 90-day post-test blood samples from six of the eight responders. The longitudinal responses of these subjects are presented in Table 1b. At each experimental period, the paired difference was significant ($p < 0.025$). Cholesterol levels of these subjects 90 days following the termination of the lemongrass oil protocol were not different from the baseline values.

Medical charts provide some basis for the segregation of the subjects into the two groups. While the body weights of the two groups of subjects did not differ, the body mass index of the responders was greater ($p < 0.05$, Table 2). The charts also suggested that responders tended to have higher serum triglyceride levels. This was confirmed upon analysis of triglycerides in blood sera which had been frozen following collection. Triglyceride levels of responders were higher at the baseline ($p < 0.05$, Table 2) and continued to be 30–40% higher than those of resisters throughout the study. One of eight responders and two of 14 resisters had HDL cholesterol levels falling in the normal range.

The subjects were continued on their drug therapy during the study. The only subject recorded as taking a lipid-lowering agent, a responder, had discontinued gemfibrozil two weeks prior to the study. The single non-insulin dependent diabetic subject, a resister, took glyburide through the study. Eight of the subjects took beta-blockers and eleven took aspirin on a daily basis. Four subjects were taking thiazide diuretics. No relationship between medications and response was indicated.

The subjects recruited for this study had all undergone coronary bypass surgery within the previous seven years. All had serum cholesterol levels in excess of 250 mg/dl three months following the surgery. They had received instruction in the AHA diet (30% fat energy, 10% energy as saturated fat, 300 mg cholesterol and total calories to achieve optimal body weight). The charts suggest a

moderate to good adherence to this diet. Nevertheless, serum lipids were not responding to the diet modification at the time the subjects were recruited (Table 2). This moderate to good adherence to the dietary protocol continued throughout the study (Table 2). The intake data shown in Table 2 are calculated from food intakes recorded during the second experimental period. According to our evaluation, the subjects as a group were successful in limiting their cholesterol intake to less than 300 mg/dl. While the subjects failed to achieve the AHA goals for fat intake, the evaluation does indicate that some restriction was practiced both in terms of percent total dietary energy (36%) and in percent energy supplied by saturated fatty acids (14.5%). Diet intakes of responders did not differ from those of resisters. The subjects maintained their body weights during the trial (Table 2).

DISCUSSION

This study tested the hypothesis that lemongrass oil, an essential oil rich in non-sterol end products of plant mevalonate metabolism, would, by suppressing mevalonate synthesis, lower the serum cholesterol levels of hypercholesterolemic men. The test subjects were recruited from a group of patients who followed a diet limited in cholesterol content under medical supervision. Within the limits of this test we recorded a fall in cholesterol which approached significance at 90 days. The subjects did not respond uniformly to the protocol; 14 subjects maintained their baseline (pretest) cholesterol levels during the treatment phase. Eight subjects experienced a lowering of cholesterol during the test phase, a lowering which was reversed on discontinuation of the protocol.

Responding subjects generally differed from the resistant subjects in being more overweight (BMI, $p < 0.05$) and having higher serum triglycerides ($p < 0.05$). Responders required 20% fewer calories (per kg) to maintain body weight. Whether or not one of these factors or a specific lipoprotein disorder was responsible for the difference in response has not been determined. We note that on testing a large group of normal subjects, McNamara *et al.* (7) found two populations, one of which compensated for changes in dietary intake of cholesterol. If the non-sterol end products of plant mevalonate metabolism suppress HMGR activity and presumably cholesterol synthesis in humans as they do in animals (4,8,9) it is not unlikely that a subject heterogeneity in response to non-sterol end products similar to that reported for cholesterol (7) exists.

In this study, lemongrass oil was given at a level approximately 40% that deemed to be the maximum acceptable dose of citral, the major constituent of lemongrass oil (10). Animals have been challenged with much greater intakes of citral. Citral administered by gavage (2.4 g/kg body wt) induced hepatic peroxisomal proliferation and cyanide-insensitive palmitoyl-CoA oxidative activity in rats (11). This clofibrate-like response to a large dose of citral failed to lower serum triglycerides. Also at high levels of intake (0.5–1.0% of diet), cyclic monoterpenes apparently alter lipoprotein metabolism as HDL cholesterol is reported to be increased (12). Cyclic monoterpenes administered by gavage (13) or fed (14) suppress hepatic HMGR activity. The reduced activity traces to a decrease in enzyme mass (13). It is our hypothesis that the fall in cholesterol level recorded for 36% of our subjects reflects an end product-suppressed HMGR activity in this hypercholesterolemic sub-population.

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