Effect of a Diet High in Vegetables, Fruit, and Nuts on Serum Lipids

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We assessed the effect of a diet high in leafy and green vegetables, fruit, and nuts on serum lipid risk factors for cardiovascular disease. Ten healthy volunteers (seven men and three women aged 33 ± 4 years [mean ± SEM]; body mass index, 23 ± 1 kg/m^2) consumed their habitual diet (control diet, 29% ± 2% fat calories) and a diet consisting largely of leafy and other low-calorie vegetables, fruit, and nuts (vegetable diet, 25% ± 3% fat calories) for two 2-week periods in a randomized crossover design. After 2 weeks on the vegetable diet, lipid risk factors for cardiovascular disease were significantly reduced by comparison with the control diet (low-density lipoprotein [LDL] cholesterol, 33% ± 4%, $P < .001$; ratio of total to high-density lipoprotein [HDL] cholesterol, 21% ± 4%, $P < .001$; apolipoprotein [apo] B:A-I, 23% ± 2%, $P < .001$; and lipoprotein (a) [Lp(a)], 24% ± 9%, $P = .031$). The reduction in apo B was related to increased intakes of soluble fiber ($r = .84$, $P = .003$) and vegetable protein ($r = -.65$, $P = .041$). On the vegetable compared with the control diet, the reduction in total serum cholesterol was 34% to 49% greater than would be predicted by differences in dietary fat and cholesterol. A diet consisting largely of low-calorie vegetables and fruit and nuts markedly reduced lipid risk factors for cardiovascular disease. Several aspects of such diets, which may have been consumed early in human evolution, have implications for cardiovascular disease prevention.

THERE HAS BEEN MUCH DEBATE over how the human diet has evolved and the extent to which present-day eating habits fit with our genetically determined nutrient requirements and metabolism. Some have argued that many chronic human diseases are the result of maladaptation to our contemporary diet and sedentary life-style. This concept forms the basis of the dietary fiber hypothesis[1] and the links made between changes in diet and health associated with the industrial revolution.[2] It is also reflected in the suggestion that consumption patterns of the Paleolithic period were more in keeping with our genetic makeup.[3] However, our aim was to move this debate further back in time before the divergence of man from other primates.[4-8] The question then arises as to what extent has evolution equipped primates, including man, to deal with diets high in saturated fat and cholesterol and low in dietary fiber.

In Western nations, cardiovascular disease is the single most common cause of human mortality. The original concern that over 25% of the adult US population had serum cholesterol levels greater than 6.2 mmol/L (240 mg/dL) prompted a major campaign to decrease saturated fat and dietary cholesterol intake.[9] Among captive great apes, premature cardiovascular disease is also a significant cause of death, and surveys have identified mean fasting serum cholesterol levels of 7.23 and 8.04 mmol/L in captive gorillas.[10] These high serum cholesterol levels have been attributed to high-calorie diets often containing meat and eggs, and a lack of space for physical activity. Similar concerns are expressed about the life-style of modern man.[11] More specifically, increased intake of fruit and vegetables has been shown to protect against cardiovascular disease[12-14] and various cancers,[15,16] including cancers that are increasing in incidence in Western societies such as prostate cancer[17] and non-Hodgkin's lymphoma.[18] As a result, agencies concerned with health have recommended increased servings of fruit and vegetables.[19,20]

The two questions we therefore wished to address were as follows: (1) To what extent could we increase the consumption of vegetables and fruit, possibly to levels that might have been eaten by an ancestor common to simians and hominids; and (2) What effect would this dietary change have on lipid risk factors for cardiovascular disease?

SUBJECTS AND METHODS

We studied 10 healthy subjects (seven men and three women) aged 33 ± 4 years (mean ± SEM) with a body mass index of 23 ± 1 kg/m^2 who were recruited from among hospital and university staff and graduate students. All subjects had normal blood lipids at baseline, except one subject with elevated serum triglyceride (5.15 mmol/L)[11] and low high-density lipoprotein (HDL) cholesterol (0.84 mmol/L).[11] The subjects were nonsmokers, and none consumed alcohol on a regular basis. None were taking medications, and none had biochemical or clinical evidence of diabetes or renal or hepatic disease. No subjects were endeavoring to lose weight, and none had shown significant weight change over the previous year. Four subjects regularly exercised, and all subjects were asked to maintain the same level of physical activity during both study periods. Three subjects were lacto-ovovegetarians.

Subjects were treated with two 2-week diets in a crossover design, one of which was the vegetable diet (seven subjects started this diet first) and the other the control diet. The control diet was the subjects' habitual diet. The control study period either directly preceded the vegetable diet or followed it after an interval of at least 5 months (7 ± 4 months) to minimize the possible carryover effect of the vegetable diet period on subsequent food selection. Body weight was measured by the investigators, and fasting blood samples and 24-hour urine output were collected before the start and at the end of week 2 of each dietary phase. In addition, subjects were also asked to weigh themselves on a daily basis during the study periods and to report any consistent trend in

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weight change so that diets and caloric intakes could be modified at the end of the first week to maintain weight.

Diet

The aim for the vegetable diet was to provide the maximum amount of green leafy vegetables (cabbage, bok choy, chard, spinach, brussels sprouts, and leeks), flowers (broccoli and cauliflower), and other low-calorie vegetables (tomatoes, red, yellow, and green peppers, zucchini, cucumbers, carrots, green beans, bean sprouts, celery, radishes, mushrooms, eggplant, and okra) that could theoretically be eaten raw. The goal for leafy vegetable consumption was set at 600 g/d. Garden peas, sweet yellow corn, and eggplant were recommended at levels of 500 g/d. When cooked, vegetables were boiled or steamed. Some foods were eaten raw such as carrots, tomatoes, green peas, fruit, and nuts. The major sources of fat, raw almonds, cashews, peanuts, and avocado, were to be eaten in limited amounts (nuts, 60 to 120 g/d). Water was the recommended beverage; however, fruit and vegetable juices and plain tea and coffee were permitted. For individuals who required milk, soya milk was allowed. Higher levels of fruit and nut consumption were advised if weight loss was noted in the first week. Subjects were given lists of foods from which to select vegetables and fruit. These were divided into three categories: foods for which a minimum set weight was required to be eaten daily, foods from which the bulk of the diet was selected, and foods (fruit and nuts) to be eaten in moderation. The only foods to contain significant amounts of starch were peas and corn.

Frozen vegetables (eg, peas in the pod, brussels sprouts, garden peas, and sweet corn) (President's Choice; Loblaw, Toronto, Ontario, Canada) and nuts were provided, whereas the remaining foods were acquired by the subjects. Subjects were provided with electronic scales on which all food was weighed before being eaten, and the weight was recorded in both the vegetable and control periods. These weighed diet histories were collected from subjects at the end of each week. Diet histories were assessed using a system based on US Department of Agriculture data,[21] which also included fiber values from our own analyses and from those of Anderson and Bridges.[22] Values for animal and vegetable protein were assigned according to the origin of the food. For composite manufactured foods, we obtained the unit weights of the ingredients from the manufacturers.

Analyses

Serum stored at −70°C was analyzed for total cholesterol, triglyceride, and HDL cholesterol after heparin manganese precipitation in a single batch using the techniques of the Lipid Research Clinics.[23] Low-density lipoprotein (LDL) cholesterol level was calculated.[24] Serum apolipoprotein (apo) A-I and B levels were measured by an enzyme-linked immunosorbent assay technique,[25] and lipoprotein(a) [Lp(a)] level was measured with a commercial enzyme-linked immunosorbent assay (Terumo medical kit; Terumo, Elkton, MD). Serum electrolytes, creatinine, urea, uric acid, and total protein and albumin were analyzed in the routine clinical chemistry laboratory using standard methods for Kodak Ektachem analyzers (Eastman Kodak, Rochester, NY).

Aliquots of 24-hour urine collections stored at −70°C were also analyzed for electrolytes, creatinine, and urea in the routine clinical chemistry laboratory using standard methods for Kodak Ektachem analyzers. C-peptide level was measured by radioimmunoassay,[26] and short-chain fatty acids (SCFA) were determined by high-performance liquid chromatography[27] after vacuum distillation.[28]

Statistics

Results are expressed as the mean ± SEM. For percentage differences, week 2 vegetable diet values are expressed as a percentage of the respective week 2 control values. Treatment differences for all serum lipid and lipoprotein values were normally distributed. The significance of these percentage differences was assessed by Student's t test for paired data (two-tailed). Assuming a standard deviation for the treatment difference in serum cholesterol of 10%, as observed in other dietary studies,[29] and setting α at .05 and β at 0.8 for the power calculation, 10 subjects would be required to detect a 10% difference in serum cholesterol. Absolute treatment differences between week 2 values were assessed using the general linear model procedure (GLM) of the SAS[30] with treatment and sex as categorical (class) variables and the baseline value of each phase as a covariate. Pearson product-moment correlations and stepwise multiple regression analyses were used to determine associations between changes in blood lipids and dietary variables.[30] Eight dietary variables were included in the multiple regression analysis: total, saturated, polyunsaturated, and monounsaturated fat, dietary cholesterol, vegetable protein, and total and soluble fiber.

RESULTS

The vegetable diet protocol was adhered to adequately over the 2-week period in that no foods were eaten that were not on the list of options. However, the mean intake of leafy vegetables was only 62% ± 19% of the minimum recommended, and more eggplant, peas, and corn were consumed than had been recommended (126% ± 7%, 141% ± 9%, and 153% ± 7%, respectively). On the vegetable diet, the mean weight of all vegetables, fruit, and nuts consumed daily was 1,783 ± 275, 713 ± 79, and 100 ± 19 g, respectively. The major difference in macronutrient profiles between the vegetable and control diets was the significantly lower saturated fat intake (9 ± 1 *v* 27 ± 4 g/d, respectively, $P < .001$), the lack of dietary cholesterol (0.2 ± 0.2 *v* 218 ± 30 mg/d, respectively, $P < .001$), and the higher fiber intake (64 ± 7 *v* 29 ± 7 g/d, respectively, $P < .001$) (Table 1). In terms of micronutrients and vitamin and mineral profiles, on the vegetable diet daily nutrient requirements were met for all nutrients except vitamins E and B_{12} and zinc. However, the intake of vitamins A, B_6, and C and folate was above the Recommended Dietary Allowance (RDA) by 450%, 218%, 1,342%, and 270%, respectively, as were levels of the remaining B vitamins (Table 2). The vegetable diet was well tolerated, although participants noted increased flatulence. All had a strong desire for starchy foods, especially bread, in addition to the peas and corn included in the diet. There was no significant weight change during the control period (−0.3 ± 0.3 kg, $P = .421$). During the vegetable period, subjects lost 0.5 ± 0.2 kg ($P = .015$), but this was not significantly different from the control weight change ($P = .525$). Subjects who lost greater than 0.5 kg in the first week were advised to increase consumption of fruit and nuts in the second week to prevent further weight loss.

Baseline (week 0) levels of serum lipids were similar for both diets (Table 3), with the exception of HDL cholesterol, which was 0.19 ± 0.04 mmol/L (7 ± 2 mg/dL, $P = .002$) lower before the control diet.

After 2 weeks, lipid and lipoprotein values on the vegetable diet were lower than the corresponding control values for total cholesterol (25% ± 3%, $P < .001$), LDL cholesterol (33% ± 4%, $P < .001$), HDL cholesterol (4% ± 4%, $P = .310$), triglyceride (20% ± 5%, $P = .005$), apo A-I (10% ± 3%, $P = .012$), apo B (30% ± 3%, $P < .001$), and Lp(a) (24% ± 9%, $P = .031$) (Table 3). In addition, the following lipid and

Table 1. Calculated Macronutrient Intake on Vegetable and Control Diets Based on 2-Week Diet Records (n = 10)

Intake	Control Diet		Vegetable Diet		P
	g/d	% of Calories	g/d	% of Calories	
kcal	2,327 ± 18		2,300 ± 18		.836
Protein	83 ± 7	14 ± 1	72 ± 9	12 ± 1	.223
Animal	44 ± 6	8 ± 1	0.2 ± 0.1	0.0 ± 0.0	.001
Vegetable	38 ± 9	7 ± 1	72 ± 8	12 ± 1	.012
Carbohydrate	313 ± 28	54 ± 2	352 ± 28	62 ± 1	.100
Fiber	29 ± 7	12 ± 3†	64 ± 7	27 ± 2†	.001
Soluble	9 ± 2	4 ± 1†	18 ± 2	8 ± 1†	.002
Insoluble	20 ± 5	8 ± 2†	45 ± 5	20 ± 2†	.001
Total fat	77 ± 7	29 ± 2	65 ± 10	25 ± 3	<.105
SFA	27 ± 4	10 ± 1	9 ± 1	4 ± 1	<.001
MUFA	28 ± 3	11 ± 1	32 ± 5	12 ± 2	.438
PUFA	16 ± 2	6 ± 0	19 ± 3	7 ± 1	.335
Cholesterol*	218 ± 30	94 ± 13	0.2 ± 0.2	0.1 ± 0.1	<.001

NOTE. Values are the mean ± SEM.
Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*mg/d and mg/1,000 kcal.
†g/1,000 kcal.

lipoprotein ratios were significantly reduced on the vegetable diet: total:HDL cholesterol (21% ± 4%, $P < .001$), LDL:HDL (30% ± 4%, $P < .001$), and apo B:A-I (23% ± 2%, $P < .001$) (Table 3). Using the GLM, these significance levels were, in general, confirmed for total ($P < .001$), LDL ($P < .001$), and HDL cholesterol ($P = .229$), triglycerides ($P = .108$), apo A-I ($P = .030$) and apo B ($P < .001$), Lp(a) ($P = .064$), and the ratios total:HDL cholesterol ($P < .021$), LDL:HDL ($P = .003$), and apo B:A-I ($P < .001$).

A number of factors including sex, previous vegetarian diet, order in which diets were fed, significant weight loss on the vegetable diet (≤0.5 kg), and caloric intake below Lipid Research Clinic (LRC) requirements on the vegetable diet were assessed to determine their influence on the treatment difference. In general, no significant effects could be attributed to these factors when they were used individually as main effects in the GLM analysis in addition to diet and baseline. Furthermore, lipid reductions tended to be similar in all subgroups (Table 4). Only in the case of HDL cholesterol and apo A-I were the effects of treatment order significant, with those who ate the vegetable diet first showing greater reductions than those who ate the vegetable diet in the second period. Despite this difference, no order effects were seen for the treatment difference in the ratios of total:HDL cholesterol, LDL:HDL cholesterol, or apo B:A-I (Fig 1).

Table 2. Mean Mineral and Vitamin Intake on the Vegetable and Control Diets in Relation to RDA

Micronutrient	RDA	Vegetable Diet		Control Diet	
		Intake	%RDA	Intake	%RDA
Calcium (mg)	800	875	109	979	122
Iron (mg)	17	24	141	21	123
Magnesium (mg)	350	800	229	315	138
Phosphorus (mg)	800	1,743	218	1,356	170
Potassium (mg)	1,875-5,625	8,691	155†	3,494	62†
Sodium (mg)	1,100-3,300	1,836	56†	2,601	79†
Zinc (mg)	15	12	80	8.1	53
Copper (mg)	<2-3	4.0	133†	1.3	43†
Manganese (mg)	<2.5-5.0	7.5	160	3.3	60
Vitamin A (RE)	1,000	4,500	450	*	
α-Tocopherol (mg)	10	2.8	30	2.2	22
Vitamin C (mg)	60	805	1,342	248	413
Thiamine (mg)	1.4	2.8	200	2.0	143
Riboflavin (mg)	1.6	2.4	150	2.0	125
Niacin (mg)	18	30	167	21	154
Pantothenic acid (mg)	<4.7	8.3	114†	4.5	106†
Vitamin B_6 (mg)	2.2	4.8	218	2.1	91
Folacin (μg)	400	1,078	270	394	98
Vitamin B_{12} (μg)	3	0	0	2.9	100

NOTE. Estimated RDA is for men 23 to 50 years old.
*Not calculated due to the presence of both retinol and β-carotene in foods.
†% of the upper level of the RDA normal range.

Simple correlation coefficients for the relation between treatment differences in total cholesterol, LDL cholesterol, and apo B with the major dietary variables are presented in Table 5. Treatment differences in total and LDL cholesterol were significantly related to differences in dietary cholesterol intake ($r = .73$, $P = .018$ and $r = .73$, $P = .018$, respectively) and soluble fiber intake ($r = -.82$, $P = .004$ and $r = -.71$, $P = .020$, respectively) between the two diets. The lower apo B level on the vegetable diet was also significantly related to the higher soluble fiber intake ($r = -.83$, $P = .003$) and vegetable protein intake ($r = -.65$, $P = .041$) (Figs 2 and 3). In terms of food associations with serum lipids, the increase on the vegetable diet in both flower and leafy vegetables was related to the reduction in the total:HDL cholesterol ratio ($r = .67$, $P = .032$ and $r = .71$, $P = .022$, respectively), and the increase in nut intake was related to the decrease in Lp(a) ($r = .71$, $P = .032$). Stepwise multiple regression analysis confirmed significant associations between treatment differences in soluble fiber and total cholesterol ($r = -.82$, $P = .004$) and apo B ($r = -.84$, $P = .003$) and between treatment differences in dietary cholesterol and LDL cholesterol ($r = .72$, $P = .018$).

The mean serum uric acid increased on the vegetable diet to a level that was significantly greater than the mean control value at the end of week 2 by 24% ± 7% ($P = .012$). Mean serum

Table 3. Serum Lipid and Lipoprotein Levels on Control and Vegetable Diets (mean ± SEM, n = 10)

Parameter	Control Diet		Vegetable Diet		% Difference, Week 2†	P‡
	Week 0	Week 2	Week 0	Week 2		
Cholesterol (mmol/L)						
Total	4.63 ± 0.27	4.90 ± 0.17	4.70 ± 0.23	3.70 ± 0.22	−24.6 ± 3.0	<.001
LDL	2.75 ± 0.23	2.87 ± 0.23	2.76 ± 0.27	1.95 ± 0.24	−33.3 ± 4.0	<.001
HDL	1.08 ± 0.06	1.19 ± 0.10	1.27 ± 0.09	1.13 ± 0.09	−4.1 ± 3.8	.310
Triglyceride (mmol/L)	1.76 ± 0.35	1.85 ± 0.45	1.48 ± 0.42	1.38 ± 0.31	−20.1 ± 5.3	.005
Apolipoproteins						
A-I	1.41 ± 0.05	1.52 ± 0.07	1.53 ± 0.06	1.36 ± 0.06	−9.6 ± 3.1	.012
B	1.09 ± 0.09	1.15 ± 0.06	1.10 ± 0.08	0.81 ± 0.06	−30.3 ± 2.9	<.001
Lp(a) (mg/dL)*	14.0 ± 3.2	14.9 ± 3.2	14.0 ± 2.9	11.1 ± 2.6	−24.2 ± 9.3	.031
Total: HDL	4.50 ± 0.46	4.44 ± 0.46	3.91 ± 0.38	3.47 ± 0.32	−20.6 ± 3.5	<.001
LDL:HDL	2.66 ± 0.31	2.58 ± 0.29	2.28 ± 0.28	1.81 ± 0.23	−29.8 ± 4.4	<.001
Apo B:A-I	0.80 ± 0.08	0.79 ± 0.07	0.73 ± 0.07	0.60 ± 0.05	−22.8 ± 2.4	<.001
Body weight (kg)	72.6 ± 5.2	72.4 ± 5.1	71.3 ± 5.2	70.7 ± 5.3	−0.6 ± 0.6§	.381

NOTE. Results are fasting values. For 3 subjects who ate the control diets in the first phase, week 2 values for the control diet are also week 0 values for the test diet. To convert values for cholesterol and triglyceride to mg/dL, multiply by 38.67 and 88.57, respectively.

*Data available only on 9 subjects.

†% difference week 2 = (week 2 vegetable − week 2 control) × 100/week 2 control).

‡For % difference week 2. A difference in HDL cholesterol of 12% would be required to detect a significant treatment difference at $P < .05$ assuming a standard deviation of 12%.

§% change week 0 − week 2 (difference).

urea levels were significantly lower at the end of the vegetable diet versus the control diet by 28% ± 7% ($P = .003$) (Table 6). No significant differences were seen in serum total protein, albumin, electrolytes, or creatinine concentrations.

No significant difference was seen in 24-hour urine volume or urinary creatinine excretion on either diet. After adjusting for 24-hour urinary creatinine output, urinary urea and sodium excretion were reduced significantly by 31% ± 6% ($P = .001$) and 42% ± 15% ($P = .020$), respectively, on the vegetable diet versus the control diet, whereas urinary formate and potassium excretion were increased by 114% ± 37% ($P = .014$) and 68% ± 22% ($P = .014$), respectively. There were no other significant treatment differences in urinary measurements or clearance values.

DISCUSSION

The vegetable diet resulted in a marked reduction in lipid risk factors for cardiovascular disease. The 33% reduction in LDL cholesterol was of similar magnitude to the lipid decrease seen with conventional drug therapy using a statin.[31] Furthermore, Lp(a) levels were reduced despite the relative resistance of this lipoprotein to conventional diet and drug therapy.[32] In epidemiological and secondary-prevention studies, consumption of vegetables, fruit,[12-14,33,34] and nuts[35] has been associated with a reduced risk of cardiovascular disease.[36] The effectiveness of these diets in modifying risk factors for cardiovascular disease may relate to the diverse range of biologically active components contained in plant foods.

A combination of low saturated fat and low cholesterol intake is likely to have contributed to the reduction observed in serum cholesterol. However, application of the Keys[37] and Hegsted[38] equations to our dietary data indicated that serum cholesterol was decreased more on the vegetable diet, by 34% (0.31 ± 0.15 mmol/L, $P = .078$) and 49% (0.39 ± 0.14 mmol/L, $P = .022$), respectively, than would be predicted simply on the basis of differences in dietary fat and cholesterol intake. It is possible that the reduction of dietary cholesterol effectively to zero may have had a greater effect than a comparable 200-mg/d reduction from a higher control level of dietary cholesterol intake.[39,40] In addition, the greater weight loss on the vegetable diet (0.4 kg in 2 weeks) or the absolute difference in weight between test and control groups at week 2 (1.7 kg) might have contributed to the

Table 4. Comparison of Percentage Differences (mean ± SEM) Between Test and Control Blood Lipids at Week 2 (test control) in Various Subgroups

Group	No. of Subjects	Cholesterol	LDL	HDL	Apo B	Apo A-I	Lp(a)
Men	7	−25.8 ± 4.2	−35.8 ± 5.5	−0.3 ± 4.7	−32.9 ± 3.8	−9.4 ± 4.0	−29.3 ± 8.6
Women	3	−21.7 ± 2.0	−27.5 ± 1.7	−12.8 ± 3.0	−24.8 ± 2.3	−10.1 ± 5.5	−14.1 ± 24.1
Vegetarian diet 1st	7	−25.7 ± 39	−32.9 ± 4.6	−6.3 ± 4.4	−30.9 ± 3.4	−10.6 ± 4.2	−16.4 ± 11.3
Vegetarian diet 2nd	3	−21.9 ± 4.8	−34.3 ± 9.3	1.1 ± 7.6	−29.3 ± 6.9	−7.2 ± 3.7	−39.8 ± 14.4
Vegetarians	3	−21.7 ± 6.8	−33.2 ± 11.0	7.8 ± 4.1	−30.7 ± 7.7	−4.1 ± 5.6	−27.0 ± 1.1
Nonvegetarians	7	−25.8 ± 3.4	−33.4 ± 4.1	−9.2 ± 3.7	−30.3 ± 3.1	−11.9 ± 3.5	−22.1 ± 14.3
Weight loss	6	−25.3 ± 2.9	−32.6 ± 4.0	−11.0 ± 3.9	−29.6 ± 2.8	−11.8 ± 3.8	−12.9 ± 13.3
No weight loss	4	−23.4 ± 6.7	−34.3 ± 8.8	6.3 ± 3.3	−31.7 ± 6.6	−6.2 ± 5.3	−38.4 ± 10.1
Below caloric requirement	6	−22.5 ± 2.2	−31 ± 4.4	−4.2 ± 4.6	−28.7 ± 3.1	−9.2 ± 3.0	−24.3 ± 14.1
Above caloric requirement	4	−27.6 ± 7.1	−35.9 ± 8.1	−3.9 ± 7.3	−33.1 ± 5.9	−10.2 ± 6.8	−24.1 ± 5.7

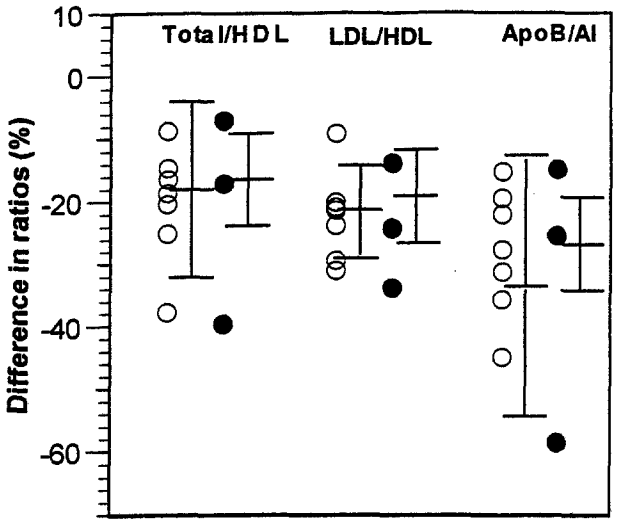

Fig 1. Reductions in the ratios of total:HDL and LDL:HDL cholesterol and apo B:A-I in subjects who ate the vegetable diet in the first (○) and second (●) periods.

greater than expected decrease in serum cholesterol. However, recent estimates of a 0.05-mmol/L decrease in serum cholesterol for every kilogram of body weight lost[41] suggest that there are still likely to be factors other than fat, dietary cholesterol, and weight differences responsible for the lower serum cholesterol value on the vegetable diet. These other factors include certain soluble fibers and vegetable proteins.

Comparative studies of lacto-ovovegetarians and vegans (who consume no animal products) have shown markedly lower serum lipids in the vegans, similar to the differences seen between the vegetable and control diets.[42] Diets composed entirely of plant foods are associated with high soluble-fiber intake. Certain types of soluble fiber have been shown to decrease serum cholesterol.[43] In the present study, differences in soluble-fiber intake were related to treatment differences in serum cholesterol and apo B. Studies of vegetarian diets have attributed some of the cholesterol decrease specifically to the

Table 5. Association Between Treatment Differences in Total and LDL Cholesterol and Apo B and Differences in the Major Dietary Variables (Pearson correlation coefficients and significance)

	Cholesterol					
	Total		LDL		Apo B	
Variable	*r*	*P*	*r*	*P*	*r*	*P*
Body weight (kg)	.11	.773	−.04	.908	−.06	.873
Caloric intake (kcal/d)	−.44	.204	−.21	.554	−.57	.085
Total protein*	−.32	.370	−.25	.479	−.42	.226
Animal	.61	.063	.50	.141	.56	.094
Vegetable	−.61	.064	−.49	.148	−.65	.041
Total fat*	.04	.914	−.12	.740	−.23	.531
Saturated	.12	.734	.25	.494	.12	.734
Monounsaturated	.07	.848	−.11	.763	−.19	.593
Polyunsaturated	−.19	.596	−.44	.209	−.33	.349
Dietary cholesterol†	.73	.018	.73	.018	.63	.053
Total dietary fiber*	−.80	.005	−.58	.079	−.79	.006
Soluble	−.82	.004	−.71	.020	−.83	.003
Insoluble	−.77	.009	−.52	.130	−.76	.012

*Macronutrients and dietary fiber expressed as g/d.
†Dietary cholesterol expressed as mg/d.

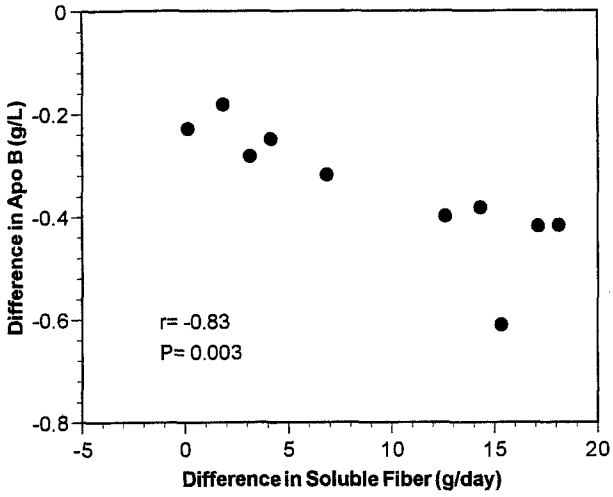

Fig 2. Relation of the 2-week treatment difference in apo B to the difference in soluble fiber intake between diets.

pectin component of soluble fiber,[44] since this viscous soluble fiber has been shown to decrease serum cholesterol.[44] Pectin has also been shown to increase urinary excretion of the SCFA formate,[45] and it is of interest that a significantly higher urinary formate output was seen on the vegetable diet. Indeed, increased SCFA generation, notably propionate, has been suggested to inhibit hepatic cholesterol synthesis.[46-48] However, at present, the effect of fiber on increasing fecal bile acid loss[49] and chenodeoxycholic acid synthesis[50,51] appears to be the best substantiated mechanism by which fiber decreases serum cholesterol.

In addition to the high fiber intake, the vegetable diet was a source of vegetable protein, which may further contribute to its hypocholesterolemic effect.[52] This possibility is supported by the negative association between vegetable protein intake and the change in apo B. Our subjects ate a mean of 100 g nuts daily. These foods contain vegetable protein and fiber and have a good dietary fatty acid profile. Consumption of 84 to 100 g nuts daily has been associated with 10% to 20% reductions in serum cholesterol.[53,54]

In relation to other risk factors for cardiovascular disease, the

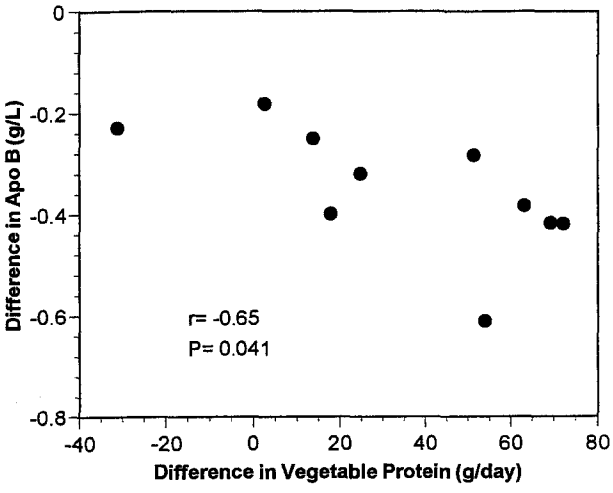

Fig 3. Relation of the 2-week treatment difference in apo B to the difference in vegetable protein intake between diets.

Table 6. Serum Urea, Creatinine, Electrolyte, and Uric Acid Concentrations on Control and Vegetable Diets (n = 10)

Parameter	Control Diet		Vegetable Diet		% Difference, Week 2*	P†
	Week 0	Week 2	Week 0	Week 2		
Urea (mmol/L)	4.8 ± 0.5	4.6 ± 0.6	4.7 ± 0.6	3.1 ± 0.4	−28.4 ± 7.1	.003
Creatinine (μmol/L)	73.2 ± 4.0	73.8 ± 4.0	79.3 ± 4.5	77.9 ± 4.0	6.4 ± 3.9	.138
Uric acid (μmol/L)	272.3 ± 25.9	275.1 ± 18.9	287.7 ± 17.0	336.9 ± 21.4	23.8 ± 7.4	.012
Potassium (mmol/L)	4.2 ± 0.04	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	−0.7 ± 2.2	.750
Sodium (mmol/L)	141.3 ± 0.4	141.0 ± 0.6	141.0 ± 0.6	140.8 ± 0.5	0.1 ± 0.5	.984
Total protein (g/L)	70.8 ± 0.8	73.2 ± 1.4	73.2 ± 1.7	72.2 ± 1.7	−2.3 ± 1.9	.258
Albumin (g/L)	44.1 ± 0.3	46.1 ± 1.3	45.2 ± 1.3	44.7 ± 1.2	−3.2 ± 2.2	.185

NOTE. Values are the mean ± SEM. To convert urea to mg/100 mL, multiply by 2.80; to convert creatinine and uric acid to mg/100 mL, divide by 88.4 and 59.48, respectively; to convert potassium and sodium to mEq/L, divide by 1.00; and to convert total protein and albumin to g/100 mL, divide by 10.

*% difference week 2 = (week 2 vegetable − week 2 control) × 100/week 2 control.

†For % difference week 2.

vegetable diet provided significant amounts of the antioxidants vitamin A (as β-carotene) and ascorbic acid, although vitamin E intake, the antioxidant for which there is the most evidence for cardiovascular disease risk reduction,[55,56] was less than the RDA. Nevertheless, the intake of vitamin E on the vegetable diet was similar to that on the control diet.

There was an unexpected but modest increase in serum uric acid levels. Frank hyperuricemia is associated with an increased risk for cardiovascular disease, especially in hyperinsulinism and gout.[57,58] However, in this study, there was no increase in 24-hour urinary C-peptide excretion to suggest an increase in insulin secretion as the reason for the higher uric acid levels.[59] Indeed, this small increase in serum uric acid may have been beneficial, since uric acid is part of the endogenous antioxidant system and may reduce the requirement for other antioxidants.[60]

Serum urea levels decreased on the vegetable diet despite adequate protein intake. The decrease in urea probably resulted from increased colonic fermentation due to increased fiber intake. Nitrogen is trapped as bacterial cell protein within the lumen of the bowel, and diffusible ammonia is converted to the less diffusible ammonium ion.[61] Both of these effects of fermentation result in increased fecal nitrogen elimination,[61] which may explain the reduced urinary urea output on the vegetable diet. This action of fiber has been used to reduce plasma urea in patients with renal failure.[62] The increased colonic fermentation resulting from the high fiber intake is supported by the increased urinary excretion of the colonically derived SCFA, acetate and formate, on the vegetable diet.

Current dietary advice for the prevention of cardiovascular disease focuses on a reduction in saturated fat and dietary cholesterol intake in calorie-controlled diets. However, by defining therapeutic diets (eg, National Cholesterol Education Program [NCEP] Step 2 diet)[11] as having less than 30% total fat, less than 7% saturated fat, and less than 200 mg cholesterol daily, a compensatory increase in consumption of plant foods is also encouraged. Some of the lipid-lowering action of these diets may result from the increase in dietary fiber and plant protein. It is possible that more specific advice on these dietary components may further increase the effectiveness of therapeutic diets in the future.

The present study has a number of limitations, including short duration and small sample size. Despite these limitations, significant reductions in lipids and lipoproteins were seen on the vegetable diet that were both significant and of large magnitude. Our previous studies with purified fiber sources have noted near-maximum reductions in LDL cholesterol even at 2 weeks.[63] Using mixed fiber sources, reductions in LDL cholesterol at 4 weeks were sustained over the following 3 months.[64] Reductions of 15% or greater in LDL cholesterol have been reported with NCEP Step 2 diets.[65] The dietary fatty acid profile of our vegetable diet exceeded minimum NCEP Step 2 requirements, with a saturated fatty acid content of less than 5% of total calories. A reduction of at least 10% to 15% in LDL cholesterol was predicted, although the observed reduction was twice this magnitude. The total number of subjects, although small, provided adequate power to detect our predicted difference. Of the other potential confounders, including inadequacy of randomization, weight gain between the first and second study periods, imbalance in the sexes, and use of vegetarians and nonvegetarians, none had a significant effect on the reduction in LDL cholesterol or the ratios of total:HDL cholesterol and apo B:A-I.

We conclude that a dietary change that eliminated animal products and greatly increased consumption of vegetables, fruit, and nuts produced a range of metabolic effects including a marked reduction in lipid risk factors for cardiovascular disease. The diet was nutritionally adequate by current standards, with the exception of vitamin B_{12} and possibly vitamin E and zinc. In evolutionary terms, these nutrients may originally have been obtained from foods eaten in small quantities and not included in this model of a high-vegetable diet. Further assessment of the overall effects of these dietary changes on health will require long-term studies with measurement of many more biochemical and physiological markers. Our original aim was to create a simian-like diet, but we did not achieve the level of leafy vegetable consumption we had hoped for. Nevertheless, aspects of the diet used here (ie, one high in plant foods) may share common features with the diet on which our simian ancestors evolved. It is hoped that the present study will encourage debate on the extent to which elements of the high-vegetable diet should be retained by contemporary *Homo sapiens* as part of the strategy for cardiovascular disease risk reduction.

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