Original Research

A Diet High in Whole and Unrefined Foods Favorably Alters Lipids, Antioxidant Defenses, and Colon Function

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Key words: phytochemicals, lipids, antioxidant defenses, plant foods

Objective: Diets rich in whole and unrefined foods, like whole grains, dark green and yellow/orange-fleshed vegetables and fruits, legumes, nuts and seeds, contain high concentrations of antioxidant phenolics, fibers and numerous other phytochemicals that may be protective against chronic diseases. This study compared the effects of a phytochemical-rich diet versus a refined-food diet on lipoproteins, antioxidant defenses and colon function.

Methods: Twelve hyperlipidemic women followed two diets for four weeks starting with a refined-food diet. Subjects then directly crossed over to the phytochemical-rich diet. Duplicate, fasting serum lipids and single, fasting antioxidant enzymes were measured at the end of the four-week refined-food diet period (baseline) and again at the end of the phytochemical-rich diet period.

Results: Total energy and total fat intake were similar during both diet periods, but there was a decrease in saturated fat (SFA) of 61% in the phytochemical-rich diet group. Dietary fiber, vitamin E, vitamin C and carotene intakes were 160%, 145%, 160% and 500% more, respectively, than during the refined-food diet period. The phytochemical-rich diet induced a drop of 13% in total cholesterol (TC) ($p < 0.05$) and 16% ($p < 0.001$) in low density lipoprotein-cholesterol (LDL-C). Erythrocyte superoxide dismutase decreased 69% ($p < 0.01$) and glutathione peroxidase dropped 35% ($p < 0.01$). Colon function was improved on the phytochemical-rich diet.

Conclusions: A diet abundant in phytochemically-rich foods beneficially affected lipoproteins, decreased need for oxidative defense mechanisms and improved colon function.

INTRODUCTION

Interest in phytochemicals, bioactive compounds found in plants, has steadily accelerated over the past several years. Phytochemically-rich foods include whole grains, legumes, fruits, vegetables, nuts and seeds and many products made from them, like oils, nut and seed butters, and baked products. A number of the biological activities of phytochemicals have been shown or hypothesized to influence a wide array of metabolic functions and disease risk. Current evidence suggests that many of these compounds are protective [1] and play important roles in preventing or delaying onset of chronic diseases and disorders such as coronary heart disease [2, 3], cancer [4], non-insulin dependent diabetes mellitus [5] and colon dysfunction [6].

For instance, dietary fibers, abundant in whole grains, legumes, vegetables, fruits, nuts and seeds, improve colon function and are as well hypothesized to help protect against heart disease and cancer [6]. Phytases, which are concentrated in the bran and the germ of whole grains, are associated with reduced risk of cardiovascular disease and cancer [7]. Tocopherols, some of which are also nutrients, are high in some nuts and seed oils, quench free radicals and help to prevent oxidative damage to protect cellular constituents [1]. Flavonoids, which occur naturally in fruits, vegetables and in some beverages such as tea and wine, reduce oxidative damage that can lead

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Journal of the American College of Nutrition, Vol. 19, No. 1, 61–67 (2000)
Published by the American College of Nutrition

to thrombosis and blockage of coronary arteries [1]. Saponins, found predominantly in legumes, have demonstrated anti-carcinogenic and hypocholesterolemic activity [8, 9]. In addition, there are hundreds of other compounds, such as tartaric acid, found uniquely in grapes, raisins and wine, which have been found to be protective against cancer [1], and the trace elements, selenium and copper, present in some nuts and whole grains as well as other foods, that play important anti-atherogenic roles in the primary catalytic defenses against metabolically generated free radicals [10].

In contrast, animal foods are naturally phytochemical-free, and the milling of grains removes more than two dozen essential nutrients, dietary fiber and many bioactive substances [11, 12]. As a result, refined and processed plant foods, like baked goods made from white flour, are largely phytochemically depleted, with the exception of the few nutrients which by law are added back. Substantial data indicate that people who eat less refined food are healthier than those that consume a diet high in refined foods [13, 14].

Much of the experimental research on the relationship between diet and chronic disease has focused on isolated dietary constituents, such as the tocopherols [1], flavonoids [1], saponins [8, 9] or dietary fibers [15]. Few investigations have examined the aggregate effect of a phytochemically-rich diet comprising whole and unrefined foods on lipids and antioxidant defenses. We set out to test the hypothesis that a diet high in phytochemicals from whole and unrefined foods can induce rapid, beneficial changes in cardiovascular risk factors, physiological measurements of oxidative defense and colon function. The purpose of this study was to compare the differential effects of a diet high in refined foods and low in fruits, vegetables, whole grains, nuts, and seeds (refined-food diet), and a diet abundant in phytochemicals from whole and unrefined plant foods (phytochemical-rich diet) on serum lipids, blood antioxidant defenses and colon function in a group of hyperlipidemic women.

MATERIALS AND METHODS

Subjects

Females 18 years or older were recruited from the San Francisco Bay Area using flyers and newspaper advertising. Subjects were excluded if they had heart disease, were taking lipid-lowering medication or had a total cholesterol ≥ 9.07 mmol/L (350 mg/dL), diabetes, gastrointestinal disorders, food intolerance or allergy or strong dietary preferences that would affect their ability to follow the study diet, or if they had been taking supplemental antioxidants or large doses of other nutritional supplements on a regular basis and were unwilling to discontinue them for one month prior to the study and during the study period. The study protocol had been approved by an independent investigational review committee and was explained to each subject who then signed an informed consent.

Experimental Protocol

This eight-week dietary trial was divided into two consecutive four-week intervention periods. During the first four weeks, subjects consumed a diet largely comprising highly

Table 1. Comparison of Daily Food Patterns Between the Two Four-Week Study Diets

Refined-Food Diet (first 4 weeks of study)	Phytochemical-rich diet (second 4 weeks of study)
Unrestricted intake	Daily consumption
White bread, pasta, pastry, snack foods	3 42-g (1-½ oz. boxes)/d sun-dried raisins*
Fast food, convenience foods	∼30 mL (2 T) almonds, hazelnuts, or pecans*
Meat, fish, poultry, eggs, dairy	∼30 mL (2 T) sesame butter*
Allowed fruit and vegetable choices and amounts:	15 mL (1 T) wheat germ oil*
1–2 servings/d selected fruit (e.g., citrus, apple, pear, banana)	250 mL (1 c) ginger tea*
1–2 servings/d selected vegetables (e.g., iceberg lettuce, green beans, peas)	500 mL (2 c) green tea*
Not allowed	2 slices whole wheat bread
Whole grains	To complete dietary intake
Dark green and most orange/yellow-fleshed fruits and vegetables	≥6 servings/d dark green and yellow/orange-fleshed fruits and vegetables
Canola, olive, and most seed/nut oils	As desired-legumes, whole grains, nonfat or 1% dairy
	84 g (3 oz.)/week meat, fish, or poultry, if desired
	Eggs (if consumed previously)
	Not allowed
	Foods made with white flour
	Convenience foods
	Whole milk dairy

* Foods provided to subjects.

refined and processed foods (refined-food diet), for which subjects provided their own food. After measurements were taken, subjects crossed over directly to the phytochemical-rich diet which was based on whole grains, legumes, fruits, vegetables, nuts and seeds, during which several foods were provided (Table 1).

Prior to starting the first four-week study period, subjects met as a group and were instructed in the study procedures and schedule and received directions for completing food records and study questionnaires. Before beginning each diet study period, subjects met again as a group or individually, as needed, with investigators and the study dietitian to receive detailed verbal and written instructions. For each study diet period, subjects were instructed in allowed and not allowed foods and were asked to focus on modifying their intake according to instructions given, while not increasing or decreasing the quantity of food typically consumed. In order to help foster compliance, subjects were not asked to adhere to a specific energy or nutrient intake level. During the refined-food diet period, subjects met once at the end of week two for at least one hour to discuss dietary issues and to monitor compliance to the study protocol. During the phytochemical-rich diet study period, subjects met weekly for at least one hour to pick up study-provided foods, to receive additional in-depth and structured dietary instruction by a registered dietitian and to discuss dietary compliance issues. Subjects were weighed prior to beginning and mid-way through each diet period. They also completed weekly food-monitoring checklists and colon function questionnaires.

Duplicate, fasting serum lipoprotein profiles and single, fasting antioxidant enzymes were assessed at the end of the four-week refined-food diet period (which was considered the baseline) and again after the four-week phytochemical-rich diet period. At each of these time points, subjects kept four-day food records of all foods and beverages consumed.

Study Diets

Table 1 shows that the two study diets were designed to contrast each other. The refined-food diet was developed to be representative of foods consumed by the average American, i.e., animal-based, high in refined and processed foods and relatively low in phytochemicals. It was also designed to be practical for subject adherence, to include enough variety to provide adequate nutrition and to be reasonably within recommendations of the USDA Food Guide Pyramid, with the exception of the number of servings from vegetables. Specifically, subjects were permitted foods from grains that were made only from white flour. Whole grains and whole grain products were not allowed. Fresh or dried fruit and juice were limited to two servings a day (serving = 125 mL [1/2 cup] or 1 medium piece) and restricted to citrus, tomatoes, pears, plums, apples, berries, green-fleshed melons and watermelon. Vegetables were limited to two servings a day (serving = 125 mL [1/2 cup] or 1 medium piece) of selected varieties, such as iceberg lettuce, summer squash, white potatoes and green beans. Dark green or yellow/orange fleshed fruits or vegetables were not permitted. There were no restrictions on meat, poultry, seafood or eggs. Subjects were permitted butter, margarine, lard, shortening and oils, except for canola, olive, other nut oils and wheat germ oil. Seeds and nuts, with the exception of commercially processed peanut butter, were not allowed.

In contrast, the phytochemical-rich diet centered around whole grains, fruits and vegetables—emphasizing dark green and orange/yellow fleshed fruits and vegetables—and was low in animal foods. Each week, subjects were provided with selected foods to consume to help foster adherence and help to insure a high intake of phytochemicals (Table 1). On a daily basis, subjects consumed three 42 g (1-1/2 oz.) boxes of sun-dried raisins, ~30 mL (2 T) each of nuts (almonds, hazelnuts, and pecans) and sesame butter, two slices of whole wheat bread, ~15 mL (1 T) wheat germ oil (for cooking or to be used as a dressing), 250 mL (1 c) ginger tea and 500 ml (2 c) green tea. Sun-dried raisins supplied quercetin and kaempferol. Green tea supplied cathechins and theaflavins, and ginger supplied gingerols and related compounds, which are all powerful antioxidants, while wheat germ, sesame seeds and nuts supplied tocopherols.

To complete their daily diets, they were to consume at least six servings (serving = 125 mL [1/2 cup] or 1 medium piece) of dark green and yellow/orange-fleshed fruits and vegetables, unlimited additional whole grains, legumes, and nonfat or 1% fat dairy products. Eggs were allowed, if eaten previously, and meat, fish and poultry were limited to 84 g (3 oz.) once a week, if desired. Convenience or altered foods, such as reduced-calorie or fat-free products were not permitted. Subjects were instructed not to consume fats other than those provided or allowed (e.g., olive oil, nut oils, and avocados were permitted), not to fry food nor or to use hard liquor. Table 2 illustrates the amount of dietary fibers and tocopherols supplied daily by selected study-provided foods that were analyzed by V-Labs (Covington, Louisiana), Alpha Laboratories (Petaluma, California) and Oregon State University (Corvallis, Oregon).

Table 2. Dietary Fiber and Tocopherol Content of Selected Study Foods in One Day's Intake

Dietary fibers (grams)	Soluble	Insoluble	Total
Sun-dried raisins	5.4	4.5	9.9
Whole wheat bread (2 slices)	<1	8	8
Nuts/sesame butter (2 T)	<1	3	3
Total	~5.5	15.5	21.9

Tocopherols (mg/100 g)	Alpha	Delta	Gamma	Total
Sesame butter (2 T)	traces	0.11	21.4	21.5
Almonds (2 T)	22.4	0.63	0.69	23.7
Whole wheat bread (2 slices)	1.7	0.1	3.3	5.1

MEASUREMENTS

Serum Cholesterol and Lipids

Total cholesterol was determined by an enzymatic method (Trinder end-point reaction) in the Abbott Spectrum Analyzer (Abbott Laboratories, North Chicago, IL). High density lipoprotein-cholesterol (HDL-C) was determined by the same method, optimized for low cholesterol concentration range, after the precipitation of the lipoproteins containing apolipoprotein B with dextran sulfate (50K MW) $CaCl^2$. True triacylglycerols (triglycerides) were analyzed by a glycerol phosphate oxidase method with endogenous glycerol blanking. LDL-C was estimated by the Friedewald equation [16]. Analytic procedures were carried out by Pacific Biometrics, Inc. (Seattle, WA) and were standardized and met performance requirements of the Liproprotein Standardization Program of the Centers for Disease Control (Atlanta, GA).

Antioxidant Enzymes

Analyses of antioxidant enzymes were conducted by the USDA-ARS, Human Nutrition Research Center, Grand Forks, North Dakota. Erythrocyte superoxide dismutase and glutathione peroxidase were measured by methods of Prohaska [17] and Rister and Bachner [18], respectively.

Diet Analysis

Baseline and four-week phytochemical-rich diet period (post-study) four-day food records were reviewed for accuracy and completeness by a registered dietitian. Food records were analyzed using the Food Processor software program, version 2.2 (ESHA Research, Salem, Oregon).

Colon Function

Changes from baseline in frequency and difficulty of elimination and stool consistency were assessed at the end of the phytochemical-rich diet period. Ratings were made on a 10-point Likert-type scale with frequency rated as 1 = less frequent to 10 = much more frequent; difficulty of elimination rated as 1 = much less effort to 10 = much more effort; and stool consistency rated as 1 = much harder to 10 = much softer. On all measures, a rating of 5 indicated no change. Improvement was defined as bowel movements being more regular and easier to eliminate without leading to loose/watery stool or diarrhea.

Statistics

The SYSTAT program (SPSS Inc., Chicago, IL) was used for statistical analyses. Baseline (at the end of the four-week, refined-food based diet period) and post-study (after four weeks on the phytochemical-rich diet) lipid values for each subject were calculated from the average of duplicate measurements at each time point. For all other variables, changes were calculated as the mean of values obtained at baseline and at the end of the study. Data were analyzed by two-tailed paired *t* test at the $p < 0.05$ level of significance. All results are reported as mean ±SD.

RESULTS

Fourteen women had entered the study. However, one subject was unable to consume all of the study-provided foods on a daily basis and withdrew during the first week of the phytochemical-rich diet period. A second subject withdrew after week two of the baseline period due to an aftereffect from the baseline blood draws. The results of this study are based on the 12 subjects who completed both four-week diet periods and measurements.

Data for laboratory measures are based on the 12 subjects, while diet data is reported for 11 subjects. Subjects' mean age was 57 ± 4.6 years old with a mean body mass index of 25 ± 4.6. Means of baseline duplicate lipid measurements were TC − 6.48 ± 0.64 mmol/L (250 ± 24.9 mg/dL), LDL-C − 4.31 ± 0.46 mmol/L (166 ± 17.9 mg/dL), HDL-C − 1.43 ± 0.36 mmol/L (55 ± 13.9 mg/dL) and triglycerides (TG) − 1.67 ± 0.87 mmol/L (147 ± 77.3 mg/dL). Baseline antioxidant enzyme concentrations were erythrocyte superoxide dismutase −0.79 ± 0.20 mcg, plasma glutathione peroxidase − 6.34 ± 1.79 EU and erythrocyte glutathione peroxidase − 3.32 ± 1.12 EU.

Review of food choices during each of the study diet periods based on food monitoring checklists and four-day food records, review of colon function questionnaires and verbal reports by subjects at study meetings indicated excellent adherence to both study diets. Evidence to support a shift to the phytochemical-rich diet was provided by comparison of baseline and post-study four-day food records. Consumption of carotenes, dietary fibers, vitamins C and E, magnesium, folate and dietary cholesterol were examined collectively as an indicator of intake of foods high in phytochemicals. With the exception of dietary cholesterol, these compounds are found

Table 3. Comparison of Micronutrient, Dietary Fiber, and Cholesterol Intake (Mean ± SD)

	Refined-Food Diet	Phytochemical-richDiet	
$n = 11$			
Carotenes (RE)	239.7 ± 256.1	1422.7 ± 702.1	$p < 0.05$
Dietary fiber (g)	14.6 ± 3.0	37.9 ± 5.5	$p < 0.001$
Vitamin C (mg)	58.9 ± 40.1	153.4 ± 75.4	$p < 0.05$
Vitamin E (mg)	12.5 ± 25.3	30.6 ± 2.6	$p < 0.05$
Magnesium (mg)	192.5 ± 86.3	347.5 ± 74.5	$p < 0.01$
Folate (mcg)	159.8 ± 73.3	250.5 ± 77.2	$p < 0.05$
Cholesterol (mg)	217.8 ± 150	27.4 ± 48.2	$p < 0.01$

abundantly in unrefined plant foods [11], especially those consumed during the phytochemical-rich diet period and, thus, should have increased substantially in comparison to intake while subjects were on the refined-foods diet. Because dietary cholesterol is found only in animal foods, it should have decreased. The data in Table 3 show an almost fivefold increase ($p < 0.05$) in carotene intake, that both dietary fiber and vitamin C increased by 160% ($p < 0.001$ and $p < 0.05$), respectively, and that vitamin E increased by 145% ($p < 0.05$). Magnesium and folate intakes increased by 81% ($p < 0.01$) and 57% ($p < 0.05$), respectively, while dietary cholesterol decreased by 87% ($p < 0.01$).

Table 4 shows that the macronutrient intake changed significantly at post-study. Shifting from the refined-food diet to the phytochemical-rich diet induced a significant 61% ($p < 0.01$) decrease in SFA and an 81% increase ($p < 0.01$) in polyunsaturated fat (PUFA) from 6.05 ± 1.59% to 11.38 ± 1.77%. Carbohydrate intake also increased significantly ($p < 0.05$) by about 20%, while protein decreased significantly ($p < 0.001$) by about 30%. Over the two study periods, the mean change in weight among subjects was not significant.

Analyses of serum lipids (Table 5) revealed a significant 13% lowering ($p < 0.05$) of TC and a 16% reduction in LDL-C ($p < 0.001$). Further, the lowering of both TC and LDL-C was universal among all subjects in this study (not shown). TC/HDL ratio was also reduced significantly ($p < 0.01$) by 14% (not shown). There were also significant reductions in measures of antioxidant defense. The erythrocyte superoxide dismutase decreased by 69% ($p < 0.01$), while the plasma glutathione peroxidase was lowered by 35% ($p < 0.01$).

Analyses of colon function questionnaires revealed improvement in all aspects measured without indication of adverse effects such as diarrhea or loose stools. Eighty-two percent of subjects reported more frequent elimination (range of ratings 7 to 10) while consuming the phytochemical-rich diet as compared to consuming the refined-food diet, while 91% of subjects reported softer stools (range of ratings 7 to 10) and less difficulty in elimination (range of ratings 7 to 10).

Table 4. Comparison of Macronutrient Intake (Mean ± SD)

	Refined-Food Diet	Phytochemical-rich Diet	
$n = 11$			
Energy (j)	6728 ± 1410	6945 ± 1088	ns
% Energy			
Total Fat	31.79 ± 10.46	30.57 ± 2.54	ns
Saturated fat	12.01 ± 6.81	4.73 ± 0.56	$p > 0.01$
Monounsaturated fat	11.16 ± 3.89	12.69 ± 1.64	ns
Polyunsaturated fat	6.05 ± 1.59	11.38 ± 1.77	$p < 0.01$
Protein	15.41 ± 4.54	11.00 ± 2.53	$p < 0.001$
Carbohydrate	49.18 ± 13.72	59.27 ± 5.59	$p < 0.05$

Table 5. Lipoprotein Profile and Antioxidant Enzymes (Mean ± SD)

	Refined-food Diet	Phytochemical-rich Diet	
$n = 12$			
Lipids (mmol/L)			
Total cholesterol	6.48 ± 0.64	5.66 ± 0.64	$p < 0.05$
LDL-C	4.31 ± 0.46	3.60 ± 0.47	$p < 0.001$
HDL-C	1.43 ± 0.36	1.45 ± 0.41	ns
Triglycerides	1.67 ± 0.87	1.35 ± 0.46	ns
Antioxidant enzymes			
Erythrocyte superoxide dismutase (mcg)	0.79 ± 0.20	0.24 ± 0.05	$p < 0.01$
Plasma glutathione peroxidase (EU)	6.34 ± 1.79	4.10 ± 1.51	$p < 0.01$
Erythrocyte glutathione peroxidase (EU)	3.32 ± 1.12	2.89 ± 0.57	$p = 0.08$

To convert cholesterol values from mmol/L to mg/dL, divide by 0.02586; to convert triacylglyerol values from mmol/L to mg/dL, divide by 0.0113.

DISCUSSION

This study was done to test the hypothesis that a phytochemically-rich diet derived from whole and unrefined foods could induce rapid, beneficial changes in lipids, in physiological measurements of oxidative defense and in colon function. Excellent protocol adherence and our results suggest that the investigation's objectives were achieved. The findings showed that a diet high in unrefined, minimally processed foods rich in phytochemicals induced a protective altering of the lipoprotein profile and an improvement in levels of oxidative enzymes and colon function relative to the refined-food diet.

To our knowledge, there is little published experimental work on the effects of whole food diets in free-living populations which has simultaneously examined both serum lipids and antioxidant defenses. The present investigation, however, does replicate similar changes in lipids that we observed in an earlier study at our center. In that project, we [19] showed that a phytochemical-rich diet produced a significant lowering of TC and LDL-C. Shifts in percent of energy from fatty acids in that study as compared with the present investigation were similar, with the exception of PUFA. Monounsaturated fat (MUFA) had significantly increased from 11% to 14% *vs.* a nonsignificant increase from 11% to 13% in this study; SFA dropped significantly from 12% to 4% of energy *vs.* 10% to 4%, respectively, while PUFA had increased nonsignificantly from 5% to 6% *vs.* a significant increase in this study from 6% to 11%. In both studies total fat was essentially unchanged from baseline at 30%.

It is clear that SFA increase both TC and LDL-C [20]. Thus the drop in SFA intake and change in polyunsaturated to saturated fat ratios in the present investigation likely explain a portion of the reductions which occurred, but according to the predictive equations of Hegsted and colleagues [20], the decrease in TC was greater than that which would have been expected based on changes in fatty acid intake alone (expected = 19.49 mg/dL *vs.* actual = 31.66 mg/dL). Hence, the abundance of bioactive substances, such as the soluble fibers found in the sun-dried raisins, legumes, and other fruits and vegetables in the phytochemical-rich diet likely also contributed to lipid lowering. However, Trowell [21] suggested that the term "dietary fiber" itself was too restrictive and that the term "dietary fiber complex" which includes "all chemical compounds naturally associated with" the structural polymers usually considered as fibers was more useful when considering relationships with refined or unrefined diets. Other phytochemicals, such as phytates and trace elements, like selenium and copper, were included, specifically among those "substances considerably reduced by modern processing."

Relative to observed changes in antioxidant defense, in theory there are only two conditions that will decrease the activity of superoxide dismutase: decreased need for antioxidant defense and decreased mineral intake. The first condition can be inferred from Milne's [22] suggestion that "conditions that produce an oxidative stress tend to increase copper-zinc-superoxide dismutase activity even during periods of low copper intake." Copper deficiency generally decreases superoxide dismutase activity [10, 23]. In the present study, activity was lower even when copper intake increased (from 0.91 $\pm$ 0.30 mg to 1.87 $\pm$ 0.39 mg [$p = 0.006$], data not shown). However, the duration of this experiment was too short to have had much of an effect on total body copper unless the phytochemical-rich diet increased copper bioavailability. Thus, we infer that the phytochemical-rich diet decreased the need for antioxidant defense; perhaps, the increase in tocopherols was helpful.

We also lowered oxidative defense indices in spite of a significantly increased intake of PUFA. This agrees with the suggestion that high PUFA levels should be protected by antioxidants against preoccupation to avoid higher risk of atherosclerosis [24]. This was evidenced by the significant increases in consumption of protective antioxidants, like tocopherols and phenolics, which were supplied by the sun-dried raisins, nuts and sesame butter. Collectively, these phytochemicals probably exerted beneficial effects by reducing various oxidative activities.

Finally, the vast improvement in colon function is highly attributable to the increased intake of plant foods which resulted in an average 160% increase in dietary fiber from 14.6 g to 37.9 g. A high fiber intake from plant foods has been shown consistently to be protective against cardiovascular disease and some cancers, and this change in intake is congruent with available evidence [25, 26].

In conclusion, to test our hypothesis we chose a diet comprising unrefined, minimally processed foods that contained not only an abundance of traditional nutrients, but also an abundance of phytochemicals, such as dietary fibers, antioxidants, phenolics and flavonoids. Individuals consuming this diet experienced lowering of TC, LDL-C and TG, had essentially no change in HDL-C, were spared the need to provide high levels of intrinsic defense against oxidative damage and reported improved colon function. These effects were likely the results of biologically active microcomponents acting additively or synergistically and not simply as singular, isolated compounds. Indeed, it may be almost impossible to isolate the pieces of the nutrition package when whole foods come with everything already properly bundled. The available evidence clearly indicates the need for more systematic research to confirm and extend these findings in larger groups and in other populations where the majority of the diet comprises refined and animal foods in order to better understand their effects on health promotion and disease prevention.

ACKNOWLEDGMENTS

Research supported by an unrestricted grant from Sun-Maid Growers of California.

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Received June 1999; revision accepted November 1999.