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ABSTRACT

The purpose of this pilot study is to compare the antioxidant protection provided by whole food supplementation when added to a random sampling of commercially available, tailored nutrients and antioxidant supplements.

Thirteen patients with degenerative diseases were evaluated for antioxidant status via blood sample testing using the FIA™ Antioxidant Profile 4000 from Spectracell Laboratories, Inc., Houston, Texas. The profile is composed of three test components: glutathione, cysteine and the FIA™ Total Antioxidant Function Test (Spectrox™) assay. Patients' baseline levels were measured while using their self-chosen ongoing regimes of various nutrients and antioxidant supplements representing some 85 commercially available products. Once their study-outset baselines were documented, patients were given a whole food supplement (Juice Plus+®) that contains phytonutrients from 17 fruits, vegetables and grains. Repeat blood samples were performed over time and compared for antioxidant levels. These were summarized and expressed as percentages with 0% representing the lowest possible antioxidant protection and 100% the highest.

All patients except one showed improved antioxidant protection after adding the whole food supplement. The overall antioxidant average before whole food supplementation was 33.05%, and 79.13% after. Average improvement was 239.7% ($p<0.005$). This pilot study suggests that whole food supplementation enhanced antioxidant protection by providing probable undiscovered components inherent in whole foods that act alone or in combination with tailored isolated single nutrients and antioxidants.

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INTRODUCTION

Americans are responding to the increasing amount of information correlating good dietary habits with prevention of disease. Advertisements from the American Cancer Society, the American Heart Association, and the National Cancer Institute recommend seven to nine servings of fresh fruit, vegetables, and grains daily to significantly reduce the risk of the leading causes of disabling, costly, and deadly diseases in this country.[1] Yet, although there is increased awareness regarding the relationship between diet, nutrition, and disease, the recommended daily servings substantially exceed what most Americans consume. Many, including this author, believe that the protective effects of fruits and vegetables come from a complex combination of known and unknown antioxidants and phytonutrients found in whole fruits and vegetables. Instead of changing their diets to obtain these beneficial ingredients, Americans are consuming more supplements designed to incorporate known antioxidant and phytonutrient cofactors found in whole foods and believed to be the "active ingredients," the beneficial components of prevention. These antioxidant

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preparations do not match nature's recipe. Because current concepts focusing on oxidative stress and free radical damage to cell physiology are powerful constructs regarding the pathogenesis of degenerative diseases,[2,3] most vitamin preparations include numerous antioxidants. The most familiar are vitamins C, E, A, beta-carotene, and selenium. As additional antioxidants with potential preventive roles in degenerative diseases are identified, they are added to commercial preparations and marketed as new and improved versions.

Three examples of such popular new antioxidants are: (1) dietary carotenoids, especially lutein, that have been included in vision-enhancing supplements to help protect against the development of macular degeneration,[4] (2) lycopene, which may be added to supplements marketed to men as part of a formulation for the prevention of prostate cancer,[5] and (3) tocotrienols, which are included in supplements marketed as enhancements for circulation.[6,7]

The implication for the consumer is that such antioxidant supplementation will protect them from oxidative damage and reduce their risk for degenerative diseases.

Americans have been conditioned to the pharmaceutical-like treatment concept of "one drug, one disease," which may be carrying over to nutritional supplementation: if penicillin prevents post-streptococcal rheumatic heart disease, then lycopene may prevent prostate cancer,[10] and carotenes may prevent digestive cancers.[11,12]

An example is the Linxian, China study[11] where isolated nutrient supplements were given to approximately 29,000 people. The objective was to reduce one of the world's highest rates of digestive tract cancers through nutritional intervention. It was also one of the world's largest cancer verses diet deficiency studies ever undertaken. At the outset, subjects were found to have low levels of at least vitamins A,C,E, carotenes, and riboflavin. The population was divided into four study groups. Each were provided one of the following nutrient combinations: vitamin C, molybdenum; retinol, zinc; riboflavin, niacin; or vitamin E, beta-carotene and selenium. No group was provided nutrient dense-whole food. Only one group showed decreased cancer rates – that of the vitamin E, beta-carotene and selenium group.

Colorectal cancer risks appear to be reduced when the carotenoid lycopene is consumed.[13] Tomatoes are a rich source of lycopene, yet when rats were grouped into those supplemented with either tomato juice or pure lycopene and exposed to the carcinogen n-methylnitrosourea, the tomato juice group showed significant protection from development of digestive tract cancer.[14] This suggests that for lycopene to be effectual, it may encompass an interdependent relationship with other phytonutrients present in the whole juice product. The dose of lycopene may have been supraphysiologic when given in an isolated fashion, disrupting some as yet unknown, undiscovered, phytonutrient balance.

This interdependence could explain why research on isolated antioxidants has yielded some disappointments in the role in cancer prevention. For example, the Harvard Physician Study of 22,000 physicians showed that 50 mg of the antioxidant beta carotene every other day was of no benefit.[15] The Ten Year Finnish Smokers Study of 29,000 smokers was prematurely interrupted at eight years because lung cancer deaths in those supplementing with beta carotene exceeded the control group.[16] The Beta-Carotene and Retinol Efficacy Trial[15] involved smokers and asbestos workers at high risk for lung cancer. Again, this study was terminated prematurely when the beta-carotene/vitamin A treatment group had *higher* death rates than the non-supplemented control group.

Some researchers[17] and clinicians believe that antioxidants and other yet undiscovered phytonutrients work synergistically within cell antioxidant systems when in appropriate concentrations. It is known, for example, that humans cannot produce vitamins C or E. Consuming vitamins C and E in supplement form or through whole foods is known to help regenerate naturally-produced antioxidants such as glutathione and lipoic acid from the oxidized to the reduced form,[17] thus helping to maintain antioxidant protection.

Practitioners are increasingly aware that all beneficial nutrients have yet to be identified and placed into supplements. Some also suspect that the molecular relationships between known and unknown antioxidants and phytonutrients, not just the quantity present, is crucial. This has been verified by studies that show that the protective effects of known antioxidants have not always yielded the expected results.

Although the recommendations are to have seven to nine servings of fresh fruit, vegetables and grains daily to significantly reduce several health risks, less than 10% of adults in the U.S. consume at least five servings of fruits and vegetables per day.[8] More disturbing is the fact that this dietary pattern has been a model for children. Studies indicate that 99% of children do not get the USDA recommended number of servings of the five food groups per day.[9]

Medical practices are beginning to emphasize the preventive role of good nutrition and lifestyle, with a patient base typically presenting with preexisting large and varied nutritional supplement programs. Patients generally seek guidance from their physician on supplements and lifestyle programs, which in part led to this study.

SUBJECTS AND METHODS

Subjects: Subjects in this pilot study were thirteen patients in the author's medical practice who qualified initially by their motivation to participate in and complete the study, including a willingness to pay for the antioxidant assays and the requisite whole food nutritional supplement.

These qualifications were established to enhance patient compliance and reduce attrition. The study group included 6 females and 7 males, with a range in age of 35 to 84, an average of 64.

The degenerative diseases represented as the patient's chief complaint, and their frequency (in parentheses as number of patients) were as follows: osteoarthritis (5), non-insulin dependent diabetes mellitus (1), insulin dependent diabetes mellitus (1), post cerebrovascular accident (1), atherosclerotic heart disease (1), fatigue (8), menopausal symptoms (1), hyperlipidema (2), hypertension (3), depression (4), insomnia (1), anxiety (1), and memory loss (1).

The nutritional and antioxidant regimens of the patients in this study were diverse, with individual contents and frequency (in parentheses as number of patients) as follows: combined total number of supplements of all patients, 85; average total number of supplements per patient, 6.5, range 1 to 16; multivitamin/mineral (10); vitamin C (9), range 500-5,000 mg/d; vitamin E (8), range 400-2,000 IU/day; selenium (4), range 200-225 mcg/d; CoQ10 (6), range 100-600 mg/d; essential fatty acids, primarily omega-3 (7), range 120-3,000 mg/d.

Other supplements included barley and other greens, probiotics, magnesium-potassium, *Ginkgo biloba*, B-complex, calcium, glucosamine sulfate, amino acids, vitamins A, B-12, and D, beta carotene, folic acid, zinc, boron, trace minerals, echinacea, green tea, flavonoid preparations, alpha lipoic acid, saw palmetto, garlic, digestive aids, and bilberry.

Study Design: This study provided for only one alteration to the patient's current nutritional supplement program: addition of a whole-food nutritional supplement derived from 17 raw fruits, vegetables, and grains. The patients were informed of this author's belief that whole food nutrition may enhance antioxidant protection by providing phytonutrients that have not yet been discovered, and therefore are possibly lacking in their current regimens.

Regarding diet, all patients were instructed to avoid refined carbohydrates, which some patients had already done. Each patient was educated about the glycemic index of foods and provided reference materials to assist them to avoid high-glycemic-index foods.

No pill count was conducted, as this study relied on patient compliance strengthened through spending their own money on the whole food supplement and the cost of the antioxidant assays.

Before the follow-up assay was performed, patient compliance was reviewed to confirm consistent daily use of the whole food supplement for a minimum of 28 days prior to repeating the assays.

MATERIALS AND METHODS

Whole Food Supplement: The phytonutrient supplement used in this pilot study was a commercially available whole food nutrient product, *Juice Plus+®* [National Safety Association, Memphis, TN], that contains phytonutrients from 17 different raw fruits, vegetables, and grains in capsule form. The recommended standard daily serving for this phytonutrient product is two fruit capsules in the morning and two vegetable capsules in the evening.

Antioxidant Assay: The antioxidant assay used to evaluate each patient's level of antioxidant status was the FIA™ (Functional Intracellular Analysis) Antioxidant Profile 1400, commercially available from SpectraCell Laboratories Inc. [Houston, TX]. The profile is comprised of three test components; glutathione, cysteine, and the FIA™ Total Antioxidant Function test (Spectrox™) assay. This antioxidant profile assay was chosen because this author believes that SpectraCell Laboratories' use of isolated lymphocytes to assess antioxidant capacity provides more specific data on antioxidant activity, in contrast to most other analyses, which utilize chemical assays of oxidation end-products or measurement of enzyme activities involved in antioxidant pathways.

The Spectrox™ assay evaluates total antioxidant function by exposing the patient's cultured lymphocytes to a free-radical-generating compound, cumene hydroperoxide. The test indicates that the greater the inhibition of lymphocyte growth, the less the patient's cells are capable of generating antioxidant activity.

Analysis of Antioxidant Assays: The analysis provided by SpectraCell Laboratories compares the results of the Spectrox™ assay with each of the GSH and cysteine tests. As an example of the analysis, a low Spectrox™ reading and a significantly higher GSH reading suggest that the patient's total antioxidant function is low but GSH function is not. As a result, nutritional intervention with a GSH precursor such as n-acetyl cysteine may not be the optimal approach to raise overall antioxidant capacity.

RESULTS

The results of the antioxidant assays for the 13 patients are listed in Table 1 and presented graphically in Figure 1. All patients except one showed improved antioxidant protection after adding the whole food supplement. The results of the antioxidant assays below are expressed as percentages, with 0% representing the lowest possible antioxidant protection identified by this assay, and 100% representing the highest.

In all but one case, the assays demonstrated a significant improvement in antioxidant protection, summarized as follows and in Figure 2:

1. Overall assay average before whole food supplementation was 33.0% (range 13.1 to 64.4).
2. Overall assay average after was 79.1% (range 37.5 to 96.3).

3. Average improvement between before and after assays was 239.7% [single-tailed t test, $t(1,26) = 0.72$, $p<0.005$].
4. Nine patients (69%) had final assays ≥ 75; their average before was 33.7% (range 13.1 to 64.4) and average after was 88.5% (range 75.6 to 96.3), yielding an average improvement of 262.6%.
5. Four patients (31%) had final assays ≥ 95; their average before was 37.7% (range 21.3 to 55.6) and average after was 95.3% (range 95.0 to 96.3), yielding an average improvement of 173.2%.
6. One patient (C) showed a 27.7% decrease in antioxidant protection by a drop of 14.4% (from 51.9 to 37.5).

Table 1. Antioxidant Assay Results (in percentage points of antioxidant protection)

Patient	Before Whole Food Supplement	After Whole Food Supplement
A	33.8	73.1
B	55.6	95.0
C	51.9	37.5
D	64.4	75.6
E	25.0	83.1
F	19.4	66.3
G	44.4	96.3
H	21.3	95.0
I	25.0	85.0
J	13.1	85.6
K	29.4	95.0
L	21.3	55.6
M	25.0	85.6

As shown in Table 1, antioxidant assays were taken before and after adding the whole food supplement to the patients' current regimens. The interval between initial and follow-up antioxidant assays ranged from 2.5 months to 8.2 months, with an average of 4.3 months.

DISCUSSION

A clear trend toward improved antioxidant protection was noted early in this study. This suggests that the growth inhibition of leukocytes in the antioxidant assay had been overcome by factors supplied by the whole food nutritional supplement in 12 of the 13 patients.

This clinical study manipulated one variable, the addition of a whole food nutritional supplement to the patients' pre-existing supplement programs. Initial observations in tracking these patients are obviously on the scale of a pilot study. However, such early, dramatic improvements in oxidative protection or reserve evidenced in these live-cell functional assays should help encourage research directed at the improved diets suggested by such

Figure 1.

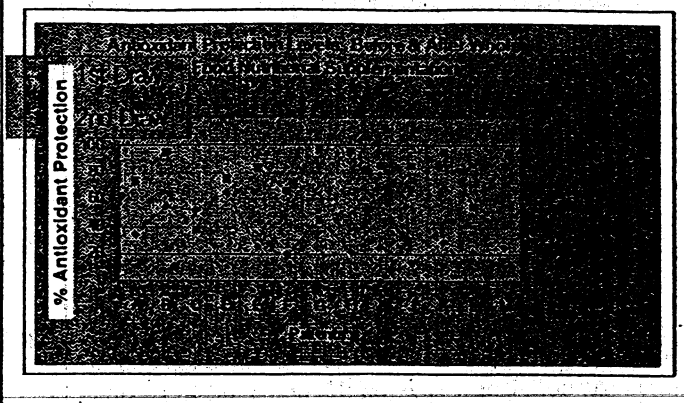
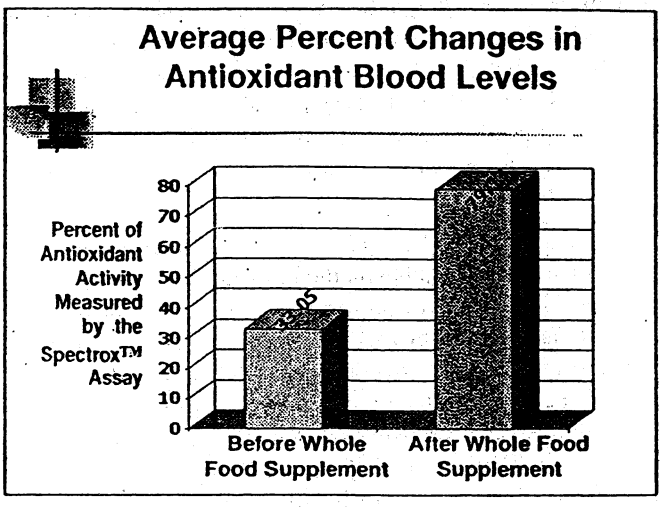


Figure 2.



organizations as the American Cancer Society and the American Heart Association.

It should be noted that in this pilot study, only patients with degenerative diseases were evaluated for their antioxidation status. Future studies should also evaluate the impact of whole food nutritional supplementation in normal healthy individuals.

The fact that the only intervention in this study was the addition of a concentration of fruits and vegetables points to the powerful impact that the poor quality of the American diet has on health. This study identifies the potential for whole food nutrition to be provided in convenient capsule form. Conversely, attempts to create commercial vitamins with only known, isolated, antioxidant components may be missing the mark.

The one patient whose antioxidant protection dropped 27.7% stands out as an indication of the complexity of the innumerable factors involved in antioxidant research. One possible explanation is that the patient's results correlated to his most significant complaint, lack of memory.

This study suggests that consumers attempting to derive antioxidant protection with pharmaceutical-like preparations

of isolated or combinational antioxidants appear to be failing, as measured by the assays of these subjects. Prevention in a convenient capsular form is their goal, but as cited earlier, some other antioxidant trials using that approach have failed to reduce the occurrence of cancer and heart disease significantly. The results of this study suggest the existence of an intricate antioxidant phytonutrient network found in whole foods, and that the antioxidants found in supplement preparations are but a subset of that larger network.

CONCLUSION

This author hypothesized that increasing patients' intake of fruits and vegetables through whole food nutritional supplementation would increase antioxidant levels in the blood. Antioxidant assays on 12 of 13 subjects substantiated this hypothesis and detailed a substantial increase in antioxidant levels. Although the study wasn't designed to control many variables affecting antioxidant protection, the author maintains that the observed overall increases were clinically significant, and justify larger, well-designed clinical trials to confirm the findings in this initial study. Also, a broader range of degenerative diseases needs to be included in future studies.

Whole food nutritional preparations in capsular form appear to be the most reasonable method of supplementation to approximate the dietary recommendations known to positively impact risk for cancer and heart disease. As stated previously, this method may offset the typical American diet, one woefully lacking in size and number of servings rich with fruits, grains, and vegetables. This study provides encouragement and hope that the future will provide some remedies, and some insurance, for lifestyles falling short in the area of balanced and protective diets. Whole food nutritional supplements may be a great stopgap measure.

ACKNOWLEDGEMENTS

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