Antioxidant Protection and Other Health Benefits of a Novel Phytonutrient Formula

Results from a clinical study demonstrated the potential of PR-581 to reduce oxidative stress, inflammation, and metabolic disturbances in healthy individuals.

INTRODUCTION

Throughout history, botanicals have been used in many cultures around the world to improve health and treat illnesses. Today, increased consumption of fruits, vegetables, herbs, and spices is associated with reduced risk of many chronic diseases.[1-3] Scientists have discovered that these plants contain a wide variety of phytonutrients (a.k.a. phytochemicals) with many health benefits. However, data from the National Health and Nutrition Examination Surveys 2003-2006 show that fewer than 10% of U.S. adults consume enough fruits and vegetables, suggesting that the majority of Americans are unlikely to fully receive the health benefits provided by phytonutrients.[4]

Researchers have found that many phytonutrients have antioxidant properties that may reduce oxidative stress.[5] Indeed, oxidative stress has been identified as an etiologic factor in aging, diabetes, coronary heart disease, and cancer. Restoration of oxidative/reductive balance has been associated with improved health outcomes in animal and human studies.[6-8] For example, the family of flavonoids have been shown to scavenge free radicals, eliminate radical precursors, elevate endogenous antioxidants, inhibit oxidative DNA adduct formation, and inhibit LDL oxidation.[9-11] Additionally, many phytonutrients confer their beneficial effects through modulation of signal transduction pathways and signal transduction molecules, leading to decreased inflammation and increased stress resistance and phase-2 detoxification capability.[12-14]

A novel formula, PR-581, has been formulated by Metagenics to help meet the needs of adults interested in supplementing their diet with both multivitamins/minerals and health-promoting phytonutrients. Consistent with a recent study showing that smaller amounts of a variety of phytonutrients have greater beneficial effects than larger amounts of fewer phytonutrients,[15] PR-581 provides a wide spectrum of concentrated phytonutrient extracts to maximize health potential. This novel blend (patent pending) was formulated according to the positive results from two laboratory assays, the Total $ORAC_{FN}$ Assay and the Comet Assay. The Total $ORAC_{FN}$ Assay is a comprehensive Oxygen Radical Absorbance Capacity assay that measures antioxidant capacity against 5 primary reactive oxygen species (peroxynitrite, hydroxyl, super oxide anion, singlet oxygen, and peroxyl) found in humans, as opposed to the traditional ORAC assay, which only captures the antioxidant capacity against peroxyl radicals. PR-581's phytochemical blend (equivalent of 1 tablet) scores an impressive range of 11,000 to 12,000. The Comet Assay measures at the individual cell level the maintenance of DNA integrity following exposure to oxidative damage,[16] and PR-581 effectively reduces oxidative damage caused by peroxide (**Figure 1**).

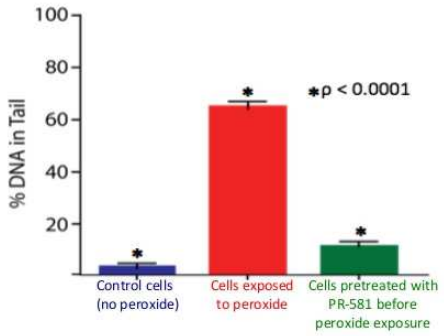

Figure 1. Data from the Comet Assay demonstrate that PR-581 pre-treatment effectively reduces oxidative damage caused by peroxide, indicating PR-581's antioxidant property.

Encouraged by these positive results, researchers at Metagenics conducted a clinical study to investigate the potential clinical benefits of PR-581.

PYM1 STUDY

Objectives. The objective of this open-label study was to examine the safety and efficacy of PR-581 in healthy adults over 4 weeks.

Primary endpoints:

- Serum carotenoids
- Vitamin B_{12}
- Folic acid
- Homocysteine levels

Secondary endpoints:

- Serum oxidized LDL (oxLDL), a marker of oxidative stress and cardiovascular risk
- Serum high-sensitivity C-reactive protein (hs-CRP), F2-isoprostane, and myeloperoxidase (MPO) levels, which are markers of inflammation
- Serum plasminogen activator inhibitor-1 (PAI-1), a marker of metabolic syndrome and obesity

Study population. Eligible participants were men and women between 18 and 65 years of age (inclusive) who were willing to maintain current lifestyle (diet and exercise) practices, to adopt the study diet, and were willing to give written informed consent to participate in the study. Main exclusion criteria included (i) use of nutritional supplements, medications, drugs, and special food plans within 2 weeks prior to the study, (ii) history of cardiovascular, renal, hepatic, mental and autoimmune disease, (iii) history of allergy or intolerance to study products, (iv) weight loss of ≥ 10% of total body weight within 6 months prior to the study, and (v) pregnancy or breastfeeding.

Study design. The study flow chart is as follows (**Figure 2**):

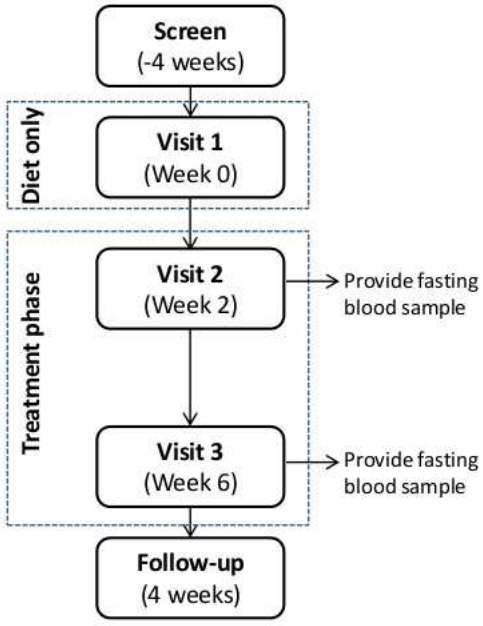

Figure 2. Study flow chart. At Visit 1 (Week 0), participants (N=15) were instructed to begin a 2-week diet only phase, which limited intake of fruits and vegetables to a total of 2 servings/day. At Visit 2 (Week 2), subjects were instructed to begin consuming 2 tablets of the nutritional supplement PR-581 every morning with a meal for the subsequent four weeks. At Visits 2 (Week 2) and 3 (Week 6), fasting serum samples were obtained for analyses, and compliance with protocol was monitored at Visit 3 by count of remaining study product and evaluation of diet diaries.

A clinical evaluation by the study investigator and adverse event collection were completed at every study visit. A follow-up visit was available four weeks after the end of the study in case there was need to follow-up on an adverse event.

Study diet: The study diet restricted food-derived antioxidants to the average daily amount reportedly consumed by Americans.[17] **Table 1** summarizes food restrictions and maximum servings of allowed foods.

Table 1. Restrictions and maximum servings of allowed food items. Numbers describe the frequencies of consumption of allowed foods during study participation.

Not allowed	Coffee, chocolate, cocoa powder, tea, red wine, fruit juices
Restricted	Beer (2 per day) or white wine (1 glass per day)
Fruit/Vegetables allowed	Apple/banana/pear (13), citrus fruit (1), beet/fennel cooked (1), zucchini/aubergines (eggplant) (2), asparagus/string beans (2), mixed salad (7), tomatoes (2), tomato sauce (2), pesto sauce (1)

Study product: PR-581 is a multivitamin/multimineral supplement with a unique phytochemical profile. The amounts of these vitamins and minerals per tablet are typical of commercially available multivitamin supplements and are provided at or below the Recommended Daily Intake (RDI) as recommended by the Institute of Medicine. Five ingredients (vitamin B_{12}, pantothenic acid, vitamin B_6, riboflavin, and thiamin) are provided at doses greater than the RDI. The dose for vitamin B_6 is less than the Tolerable Upper Intake Level; these limits are not established for vitamin B_{12}, pantothenic acid, riboflavin, and thiamin. Botanical based compounds and extracts include acacia, artichoke, blueberry, mixed carotenoids, cinnamon, grape seed, green coffee bean, green tea, hesperidin, *Momordica chiarantia*, *Polygonum cuspidatum*, pomegranate, prune, rosemary, and watercress.

Statistical analysis Serum biochemical markers and lipid profile were analyzed using the paired t-test platform in Microsoft Excel. *P*-value compared baseline value (Week 2) to end of study (Week 6), and $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics. Of the 15 participants enrolled in the study, 8 were women and 7 were men. Their average age (mean ± SD) was 41.7 ± 14.9 years old, and their body mass index (BMI) was 28.0 ± 5.6 kg/m^2. Systolic blood pressure was 117.5 ± 9.4 mm Hg and diastolic blood pressure was 73.5 ± 8.5 mm Hg.

Primary endpoints. After 4 weeks of supplementation with PR-581, serum concentration of carotenoids, folate, and vitamin B_{12} were significantly increased compared to baseline.

Homocysteine was unchanged (**Table 2**).

Table 2. Summary of primary endpoints. Data are expressed as mean±SE.

Variable	Week 2 (Visit 2)	Week 6 (Visit 3)	P value
Carotenoids (µg/dl)			
cis-lycopene	6.83 ± 1.04	10.81 ± 1.31	**<0.001**
trans-lycopene	8.71 ± 1.07	11.73 ± 1.24	**<0.01**
lutein	13.78 ± 1.97	16.32 ± 2.33	**<0.05**
zeaxanthin	4.56 ± 0.66	16.93 ± 1.66	**<0.001**
α-carotene	5.48 ± 1.17	7.7 ± 1.51	**<0.01**
β-carotene	16.77 ± 3.1	40.12 ± 6.04	**<0.001**
β-cryptoxanthin	8.85 ± 1.22	18.44 ± 1.81	**<0.001**
Folate (ng/ml)	12.5	20.5	**<0.001**
Vitamin B_{12} (pg/ml)	640.1	738.4	**<0.01**
Homocysteine (µM/l)	7.8 ± 0.62	8.0 ± 0.74	0.375

Secondary endpoints

Oxidized LDL: A significant reduction in oxidized LDL-C was observed from 54.0 ± 3.3 U/L at Visit 2 to 45.0 ± 2.9 U/L at the end of the intervention (**Figure 3**).

PAI-1: PAI-1 also was significantly reduced at Week 6 (4499 ± 194 pg/ml) compared to Week 2 (5914 ± 243 pg/ml).

MPO: Similarly, MPO was significantly reduced as a result of the PR-581 intervention, from 236 ± 24 µg/l at Visit 2 to 165 ± 21 µg/l at Visit 3.

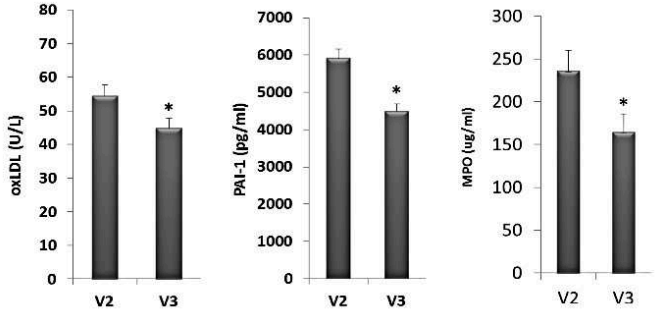

Figure 3. Oxidized LDL, PAI-1, and MPO were analyzed at Visit 2 and Visit 3. *P<0.01.

The hs-CRP and F2-isoprostane levels did not change significantly after the PR-581 intervention.

Safety evaluation. No serious adverse events were reported. The most common adverse event was mild nausea, which occurred when the product was taken without food.

DISCUSSION

This study demonstrated that PR-581 is safe and effective in healthy subjects over a 4-week period. PR-581 is well absorbed, as indicated by the increased circulating carotenoid levels as well as those of folate and vitamin B_{12}.

Carotenoids such as α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin have many important physiological functions in the human body. They act as antioxidants and to absorb UV light, protecting the skin and eyes from damage.[18-19] Evidence has shown that the amount of macular pigment, composed of lutein and zeaxanthin, is inversely associated with the incidence of age-related macular degeneration.[20] Epidemiological studies have suggested a potential role of β-cryptoxanthin as an osteogenic factor in preventing osteoporosis in human subjects.[21] Lycopene is one of the most potent in vitro antioxidants, and blood lycopene concentrations are associated with decreased cardiovascular disease and prostate cancer risk.[22] Scientific literature on the role of carotenoids in cardioprotection, chemoprevention, DNA stability, and healthy aging is expanding rapidly every day.[23-25]

Environmental toxins such as cigarette smoke and dietary carcinogens, as well as endogenous production of reactive oxygen species due to normal metabolism can lead to excess exposure to oxidative stress throughout the body. Long-term exposure to oxidative stress can permanently damage cells and tissue, and has been identified as a contributing factor to the progression of cardiovascular disease, rheumatoid arthritis, and cancer, among other chronic illnesses.[26-28] Numerous phytonutrients act as antioxidants, protecting cells from oxidative stress by neutralizing free radicals.[29-31]

Oxidative modification of LDL and the formation of oxLDL is involved in the pathogenesis of atherosclerosis, and oxLDL levels correlate with cardiovascular events.[32] Research has suggested that oxLDL levels could be a useful marker for cardiovascular risk.[33] MPO, a member of the heme peroxidase superfamily that is released upon leukocyte activation, has been found to reflect endothelial dysfunction, inflammation, atherosclerosis, and oxidative stress,[34-35] suggesting its value as a predictor of cardiovascular disease in certain populations.[36] PAI-1, secreted by adipose tissue, has been found to increase in individuals affected by obesity and type 2 diabetes.[37-38] Studies have shown that PAI-1 may increase cardiovascular risk and its serum levels are predictive of incident of cardiovascular disease.[39] Furthermore, PAI-1 could act as an inflammatory mediator that negatively impacts cardiovascular health.[40] Reductions of these biomarkers observed at the end of the 4-week study suggest that PR-581 may have the potential in improving certain markers of inflammation, oxidative stress, and cardiovascular disease.

As part of a healthy lifestyle including regular physical exercise and healthy dietary patterns, for example, such as the Mediterranean diet, daily supplementation with PR-581 can provide additional phytonutrients that support the body's antioxidant and detoxification mechanisms, as well as cardiovascular health.

CONCLUSION

Supplementation with PR-581 significantly increased serum carotenoids, vitamin B_{12}, and folate levels compared to baseline. Furthermore, a reduction in oxidized LDL-C was observed. Also, certain biomarkers of oxidative stress and cardiovascular risk (e.g., MPO) and inflammation, insulin resistance, and obesity (e.g., PAI-1) were improved. No serious adverse events related to the study product were reported, and clinical assessments of safety produced no findings indicative of toxicity. This study demonstrates that PR-581 is safe and suggests that it provides support for cellular antioxidant protection and cardiovascular health.

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