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Comparison of a Broad Spectrum Anti-Aging Nutritional Supplement with and without the Addition of a DNA Repair Enhancing Cat's Claw Extract

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ABSTRACT

Dietary supplements, designed to fortify the antioxidant status of human metabolism, are intended to provide nutritional support against the aging process. The strategy is based on the observation that environmental and metabolic sources of oxidatively generated free radicals damage key macromolecules, such as DNA, that in turn alter physiologic processes, and in turn may contribute to aging and age-related diseases such as cancer, as well as cardiovascular and immunologic disorders. This study investigates whether a commercially available, broad spectrum anti-oxidant formulation containing 12 vitamins, 8 minerals, 2 agents to provide "blood sugar/insulin support," 3 botanical antioxidants, one methylating factor, two "fat metabolizers," an "absorption enhancer," a "brain enhancer," a "whole food" ingredient, 2 "cellular energizers," a nucleotide precursor, 2 amino acids, a fatty acid complex, a "probiotic complex," and a digestive enzyme (formula one) could be improved through inclusion of an ingredient that enhances DNA repair (C-Med-100). The two formulations were compared using four intermediate endpoint biomarkers: 8-OH guanine DNA adducts, serum thiols, and Interleukins 1a and 1. Whereas both were shown to be effective at reducing DNA damage, the second, more inclusive formulation appeared to be more effective.

INTRODUCTION

THE POTENTIAL BENEFITS of antioxidant nutritional supplementation have moderate scientific support as well as broad acceptance in the marketplace and are the driving force in this billion dollar industrial sector (U.S. Pharmacopeia Dispensing Information, 15th edition, 1995).[1] Despite these considerations, there has been a lack of clinical studies demonstrating that such supplementation can be used as a beneficial dietary intervention in human patients.

Environmental and metabolic oxidants produce oxygen-centered radicals that damage signal-transducing macromolecules (e.g., DNA), and this damage may, in turn, contribute to age-associated disorders such as cancer, autoimmunity, and inflammation.[2] The mechanisms leading to such diseases, however, constitute a complex, multi-step process. It has proven both

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Dr. Vince Giampapa is CSO of Optigene-x, the company developing and manufacturing Optigene Professional for the marketplace. Dr. Aristo Vojdani is CEO of Immunosciences Lab, Inc which is the company that provided the DNA adduct determinations. Dr Ronald W. Pero is CEO of Campamed, LLC which is the company that holds the patent rights to the thiol test. Drs. Giampapa, Vojdani and Pero all own significant stock in their respective companies but not in each others.

TABLE 1. THE INGREDIENTS AND DOSES ADMINISTERED THREE TIMES DAILY IN AM, MIDDAY (MD), AND PM FOR OPTIGENE PROFESSIONAL

Optigene Pro AM Formula

Supplement Facts
Serving Size: 4 Caplets (1 AM Daily Pack)
Servings Per Container: 28 (4 Week Supply)

	Amount Per Serving	% Daily Value
VITAMINS		
Vitamin A (as retinyl palmitate and 85% as beta-carotene)	3,500 IU	70
Vitamin C (as ascorbic acid and ascorbyl palmitate)	200 mg	333
Vitamin D (as cholecalciferol)	67 IU	17
Vitamin E (as d-alpha tocopheryl succinate and from mixed natural tocopherols)	100 IU	333
Thiamin (as thiamin HCl)	10 mg	667
Riboflavin (as riboflavin and riboflavin-5-phosphate)	1 mg	59
Niacin (as niacinamide and niacin)	125 mg	625
Vitamin B_6 (as pyridoxine HCl and pyridoxal 5-phosphate)	25 mg	1250
Folate (as folic acid)	100 mcg	25
Vitamin B_{12} (as cyanocobalamin)	150 mcg	2,500
Biotin	100 mcg	33
Pantothenic acid (as D-calcium pantothenate)	25 mg	250
MINERALS		
Iodine (as potassium iodide and from kelp)	50 mcg	33
Calcium (as calcium carbonate)	500 mg	50
Zinc (from Multimins™† and as zinc glycinate)	4 mg	27
Selenium (as selenomethionine and from Multimins™†)	60 mcg	86
Copper (as copper lysinate and from Multimins™†)	0.4 mg	20
Manganese (as manganese gluconate and from Multimins™†)	0.4 mg	20
Chromium (as chromium polynicotinate and from Multimins™†)	100 mcg	83
Molybdenum (from Multimins™† and as sodium molybdate)	20 mcg	27
Sulfur (from Multimins™†)	2.5 mg	*
BLOOD SUGAR/INSULIN SUPPORT - Proprietary Blend of Natural Herbs and Minerals		
Vanadium (as vanadyl sulfate and from Multimins™†)	50 mcg	*
Fenugreek seed, Alpha-lipoic acid & Coenzyme Q-10	75 mg	*
BOTANICAL ANTIOXIDANTS		
Green tea leaf extract (40% catechin and polyphenols)	100 mg	*
Turmeric rhizome extract (95% curcuminoids)	50 mg	*
Ginkgo biloba leaf extract (24% ginkgo flavonglycosides, 6% sesquiterpene lactones)	100 mg	*
METHYLATING FACTORS		
Betaine HCl	8 mg	*
DNA REPAIR		
C-Med-100® (Patented extract of *Uncaria tomentosa*, standardized to 8% Carboxy alkyl esters)	150 mg	*
FAT METABOLIZERS		
L-Carnitine L-tartrate	100 mg	*
Acetyl-L-carnitine	75 mg	*
ABSORPTION ENHANCERS		
Phosphatidyl choline (from soy lecithin)	50 mg	*
BRAIN ENHANCERS		
DMAE bitratrate	50 mg	*
WHOLE FOODS - Proprietary Blend of Algaes and Herbs		
Blue-green algae, Spirulina algae & Green barley grass	250 mg	*
CELLULAR ENERGIZERS		
Cordyceps sinensis fungus extract (1% cordycepic acid)	25 mg	*
Royal jelly 3X (5% 10-HDA)	25 mg	*
NUCLEOTIDES PRECURSORS FOR GENE EXPRESSION		
Ribonucleic acid (from yeast)	100 mg	*
AMINO ACIDS		
Taurine	50 mg	*
N-Acetyl-L-cysteine	25 mg	*
FATTY ACID COMPLEX		
Omega III complex (7.5% eicosapentaenoic acid and docosahexaenoic acid, from fish body oil)	300 mg	*
PROBIOTIC COMPLEX		
Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium bifidum and Lactobacillus casei	100 million CFU	*
DIGESTIVE ENZYMES - Proprietary Blend		
Amylase, Neutral protease, Lactase, Lipolytic activity and Cellulolytic activity	1,760 units	*

* Daily value not established.

Other ingredients: microcrystalline cellulose, croscarmellose sodium, stearic acid, calcium silicate, magnesium stearate, silica, and film coating (hydroxypropyl methylcellulose, hydroxypropyl cellulose, and polyethylene glycol).

Optigene Pro PM Formula

Supplement Facts
Serving Size: 4 Caplets (1 PM Daily Pack)
Servings Per Container: 28 (4 Week Supply)

	Amount Per Serving	% Daily Value
VITAMINS		
Vitamin A [as retinyl palmitate and 85% as beta-carotene with natural mixed carotenoids (alpha-carotene, beta-carotene, cryptoxanthin, zeaxanthin and lutein)]	2,300 IU	46
Vitamin C (as ascorbic acid and ascorbyl palmitate)	165 mg	275
Vitamin D (as cholecalciferol)	44 IU	11
Vitamin E (as d-alpha tocopheryl succinate and with mixed natural tocopherols)	65 IU	217
Vitamin K (as phytonadione)	6.5 mcg	8
Thiamin (as thiamin HCl)	0.65 mg	43
Riboflavin (as riboflavin and riboflavin-5-phosphate)	10 mg	588
Niacin (as niacinamide and niacin)	140 mg	700
Vitamin B_6 (as pyridoxine HCl and pyridoxal 5-phosphate)	3 mg	150
Folate (as folic acid)	65 mcg	16
Vitamin B_{12} (as cyanocobalamin)	200 mcg	3,333
Biotin	65 mcg	22
Pantothenic acid (as D-calcium pantothenate)	32 mg	320
MINERALS		
Magnesium (as magnesium glycinate)	265 mg	66
Zinc (from Multimins™† and as zinc glycinate)	2.5 mg	17
Selenium (as selenomethionine and from Multimins™†)	40 mcg	57
Copper (from Multimins™† and as copper lysinate)	0.2 mg	10
Manganese (from Multimins™† and as manganese gluconate)	0.2 mg	10
Chromium (as chromium polynicotinate and from Multimins™†)	65 mcg	54
Molybdenum (from Multimins™†)	12 mcg	16
Sulfur (from Multimins™†)	1.5 mg	*
BLOOD SUGAR/INSULIN SUPPORT - Proprietary Blend of Natural Herbs and Minerals		
Vanadium (as vanadyl sulfate and from Multimins™†)	32 mcg	*
Fenugreek seed, Alpha-lipoic acid & Coenzyme Q-10	50.5 mg	*
BOTANICAL ANTIOXIDANTS		
Lycopene [tomato extract (20% lycopene)]	16 mg	*
Rosemary 4:1 extract (aerial parts)	6.5 mg	*
Pycnogenol (pine tree bark extract)	3.3 mg	*
METHYLATING FACTORS		
Betaine (as betaine HCl)	5 mg	*
DNA REPAIR		
C-Med-100® (Patented extract of *Uncaria tomentosa*, standardized to 8% Carboxy alkyl esters)	100 mg	*
FAT METABOLIZERS		
L-Carnitine L-tartrate	65 mg	*
ABSORPTION ENHANCERS		
Phosphatidyl choline (from soy lecithin)	32 mg	*
BRAIN ENHANCERS		
Inositol	100 mg	*
Kava kava root standardized extract (30% kavalactones)	65 mg	*
CELLULAR ENERGIZERS		
Cordyceps sinensis fungus extract (1% cordycepic acid)	16.5 mg	*
NUCLEOTIDES PRECURSORS FOR GENE EXPRESSION		
Ribonucleic acid (from yeast)	65 mg	*
AMINO ACIDS - Proprietary Blend of Essential and Non-Essential Amino Acids and Fatty Acids		
L-Arginine (as L-arginine HCl), Omega-III fish body oil complex (4.5% EPA, 3% DHA), L-Ornithine (as L-ornithine HCl), Taurine and N-Acetyl-L-cysteine	813.5 mg	*
PROBIOTIC COMPLEX		
Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium bifidum and Lactobacillus casei	65 million CFU	*
DIGESTIVE ENZYMES - Proprietary Blend		
Amylase, Neutral protease, Lactase, Lipase & Cellulase	1,168.5 units	*

* Daily value not established.

Other ingredients: dicalcium phosphate, microcrystalline cellulose, croscarmellose sodium, stearic acid, calcium silicate, magnesium stearate, silica, and film coating (hydroxypropyl methylcellulose, hydroxypropyl cellulose, and polyethylene glycol).

Optigene-F1 is identical to Optigene Professional except minus the C-Med-100 ingredient (total daily dose of 350 mg/day). These supplements have been administered as recommended for a 4-week period in this study. Optigene-x (Morristown, NJ) has formulated these products as a broad spectrum anti-aging therapeutic treatment, and they are currently being distributed by the same company.

TABLE 1. THE INGREDIENTS AND DOSES ADMINISTERED THREE TIMES DAILY IN AM, MIDDAY (MD), AND PM FOR OPTIGENE PROFESSIONAL (*CONTINUED*)

Optigene Pro PM Formula

Supplement Facts
Serving Size: 4 Caplets (1 PM Daily Pack)
Servings Per Container: 28 (4 Week Supply)

	Amount Per Serving	% Daily Value
VITAMINS		
Vitamin A [as retinyl palmitate and 85% as beta-carotene with natural mixed carotenoids (alpha-carotene, beta-carotene, cryptoxanthin, zeaxanthin and lutein)]	2,300 IU	46
Vitamin C (as ascorbic acid and ascorbyl palmitate)	165 mg	275
Vitamin D (as cholecalciferol)	44 IU	11
Vitamin E (as d-alpha tocopheryl succinate and with mixed natural tocopherols)	65 IU	217
Vitamin K (as phytonadione)	6.5 mcg	8
Thiamin (as thiamin HCl)	0.65 mg	43
Riboflavin (as riboflavin and riboflavin-5-phosphate)	10 mg	588
Niacin (as niacinamide and niacin)	140 mg	700
Vitamin B_6 (as pyridoxine HCl and pyridoxal 5-phosphate)	3 mg	150
Folate (as folic acid)	65 mcg	16
Vitamin B_{12} (as cyanocobalamin)	200 mcg	3,333
Biotin	65 mcg	22
Pantothenic acid (as D-calcium pantothenate)	32 mg	320
MINERALS		
Magnesium (as magnesium glycinate)	265 mg	66
Zinc (from Multimins™† and as zinc glycinate)	2.5 mg	17
Selenium (as selenomethionine and from Multimins™†)	40 mcg	57
Copper (from Multimins™† and as copper lysinate)	0.2 mg	10
Manganese (from Multimins™† and as manganese gluconate)	0.2 mg	10
Chromium (as chromium polynicotinate and from Multimins™†)	65 mcg	54
Molybdenum (from Multimins™†)	12 mcg	16
Sulfur (from Multimins™†)	1.5 mg	*
BLOOD SUGAR/INSULIN SUPPORT - Proprietary Blend of Natural Herbs and Minerals		
Vanadium (as vanadyl sulfate and from Multimins™†)	32 mcg	*
Fenugreek seed, Alpha-lipoic acid & Coenzyme Q-10	50.5 mg	*
BOTANICAL ANTIOXIDANTS		
Lycopene [tomato extract (20% lycopene)]	16 mg	*
Rosemary 4:1 extract (aerial parts)	6.5 mg	*
Pycnogenol (pine tree bark extract)	3.3 mg	*
METHYLATING FACTORS		
Betaine (as betaine HCl)	5 mg	*
DNA REPAIR		
C-Med-100® (Patented extract of *Uncaria tomentosa*, standardized to 8% Carboxy alkyl esters)	100 mg	*
FAT METABOLIZERS		
L-Carnitine L-tartrate	65 mg	*
ABSORPTION ENHANCERS		
Phosphatidyl choline (from soy lecithin)	32 mg	*
BRAIN ENHANCERS		
Inositol	100 mg	*
Kava kava root standardized extract (30% kavalactones)	65 mg	*
CELLULAR ENERGIZERS		
Cordyceps sinensis fungus extract (1% cordycepic acid)	16.5 mg	*
NUCLEOTIDES PRECURSORS FOR GENE EXPRESSION		
Ribonucleic acid (from yeast)	65 mg	*
AMINO ACIDS - Proprietary Blend of Essential and Non-Essential Amino Acids and Fatty Acids		
L-Arginine (as L-arginine HCl), Omega-III fish body oil complex (4.5% EPA, 3% DHA), L-Ornithine (as L-ornithine HCl), Taurine and N-Acetyl-L-cysteine	813.5 mg	*
PROBIOTIC COMPLEX		
Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium bifidum and Lactobacillus casei	65 million CFU	*
DIGESTIVE ENZYMES - Proprietary Blend		
Amylase, Neutral protease, Lactase, Lipase & Cellulase	1,168.5 units	*

* Daily value not established.

Other ingredients: dicalcium phosphate, microcrystalline cellulose, croscarmellose sodium, stearic acid, calcium silicate, magnesium stearate, silica, and film coating (hydroxypropyl methylcellulose, hydroxypropyl cellulose, and polyethylene glycol).

difficult and costly to provide supportive data for the clinical efficacy of such antioxidant therapies.

One approach is to combine many known or suspected antioxidant nutritional factors into a single inclusive formulation, in an effort to demonstrate clinical benefit even if some component factors were ineffective. This approach has been termed nutritional "therapy" as opposed to "supplementation." The downside, however (beyond increased cost), is that some nutrients may compete metabolically.[3–5] Random combination may therefore be counterproductive. Care must be taken to avoid conflicting interactions.

Theoretically, selection might favor competition between nutrients with shared mechanisms during their intestinal absorption. Electrophilic scavengers, for example, vitamin C, polyphenols, and carotenoids, might be more competitive with each other than with enzymatic scavengers, such as superoxide dismutase and catalase. Theoretical arguments notwithstanding, any formulation, however inclusive, might demonstrate competitive absorption and subsequent suboptimal antioxidant effects. There is no substitute for testing and comparison of differing formulations. This study concentrates on the application of potential intermediate biomarkers in order to determine the clinical effectiveness of two different broad spectrum antioxidant supplements.

The first formula (Optigene F1) is a broad spectrum nutritional therapy designed and marketed as an "antiaging treatment," containing 39 ingredients (Table 1). The formulation was assembled with the intent of avoiding potentially competing antioxidant mechanisms and of insuring a balanced nutritional support. Although containing a broad spectrum of nutritional components, the first formulation lacks any component known to enhance DNA repair. DNA repair directly mitigates the accumulation of oxidant-induced DNA damage and should therefore lower the risk of mutation and the resulting health consequences.[2,6–8]

To our knowledge, the second formula (C-Med-100) is the only known formulation containing a factor that might reasonably enhance DNA repair, thereby avoiding genetic damage, which might lead to disease. This ingredient is a proprietary, water-soluble—claimed to be "100% bioavailable" (CampaMed, LLC, New York, NY)—factor extracted from Uncaria tomentosa (Cat's Claw). Comparable water extracts have been shown to inhibit nuclear transcription factor–beta (NF-kB), induce apoptosis, stimulate immunity, immune cell growth

and half-life, vaccination response, and DNA repair in several species, including humans.[9–16]

Here we compare these two formulas to (1) evaluate the efficacy of a nutritional supplement in reducing oxidant-induced DNA damage in humans; (2) evaluate the use of an additional putative "DNA repair enhancer" to increase the efficacy of such supplementation; and (3) measure the correlation between a panel of different biochemical indicators of *in vivo* DNA damage, and comment on their utility as markers for "anti-aging" therapies.

MATERIALS AND METHODS

Study design

Ten healthy females and nine healthy males, ages 35–55 years, were randomly assigned to either supplemental formula. The study was originally designed as a crossover study, in which the subjects were to be supplemented for 4 weeks, the supplement discontinued for 2 weeks, and then the two groups crossed-over for an additional 4 weeks. Heparinized peripheral blood samples were collected from the subjects before supplementation, after 4 weeks of supplementation, and 2 weeks after final washout. Plasma interleukin 1a, plasma interleukin 1b, 8-hydroxy guanine DNA adducts (8-OH adducts), and plasma thiols (in the 0-80% ammonium sulfate precipated protein fraction) were measured for each patient. The gold standard for evaluation of DNA damage using these biomarkers was the starting level of (8-OH) DNA adducts. Hence, in order to measure any possible reduction in DNA damage from nutritional intervention, all DNA adduct levels <2 adducts per 10^9 DNA bases at the start of treatment were excluded.

Because the biomarkers were not determined until after the patients had been randomized to one arm or the other, eight subjects of the 19 enrolled had to be excluded either because of having levels of DNA adducts which were too low at the start of the study or because they failed to comply with the treatment regimen. In an optimal crossover design, subjects should be shown to have no measurable residual effect from the first treatment before they begin the second, crossed-over treatment. Measurements of the biomarkers were therefore completed before beginning the second, crossed-over treatment. Our data showed significant increases in the DNA adducts even after 2 weeks of washout when compared to pretreatment levels. The cross over design was therefore abandoned and before, after, and 2-week washout values were

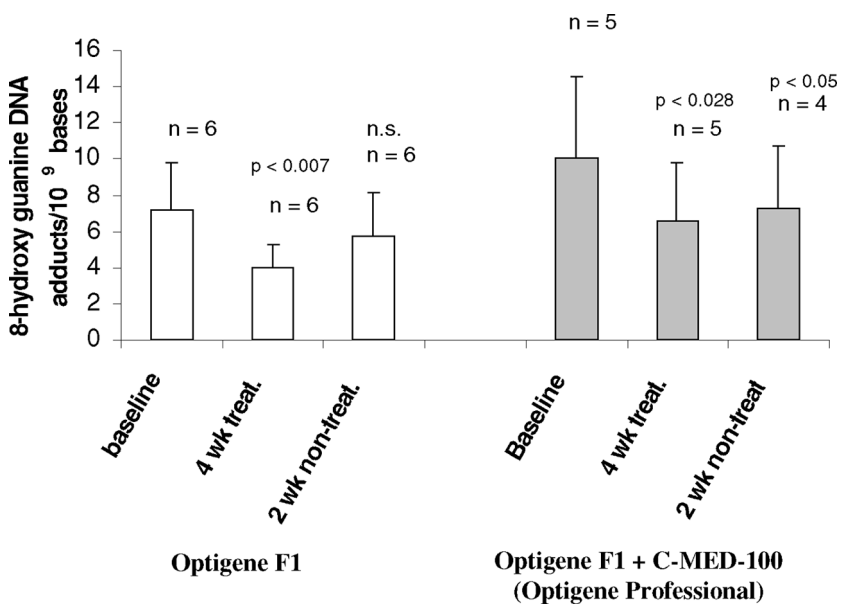

FIG. 1. DNA damage estimated by the presence of (8-OH) guanine adducts per 10^9 DNA bases in peripheral lymphocyte DNA isolated from blood samples collected before (baseline), 4 weeks after supplement (treatment), and after 2 weeks more at washout (non-treatment). Mean SD shown and comparisons made by paired *t* test to before-treatment values.

compared by paired *t* test analyses instead. After this design modification, three males and eight females satisfied the inclusion criteria and were evaluated in the study. Subjects were encouraged not to change their diet during the experiment nor to take any supplements other than those prescribed in this study.

Medical examination

A subjective questionnaire was developed for this study containing demographic data, medical history, and medications in use. Subject medical status during the experiment was evaluated by examination (by V.G.) at 4 weeks after supplement and at the end of the 2-week washout period. Informed consent was obtained from each subject. The study was conducted in accordance with the standard recommendations (the Declaration of Helsinki) guiding physicians in pharmaceutical research involving human subjects. This study was conducted under the direct medical supervision of one of the authors (V.G.). No human subjects committee reviewed this study.

Dosage

Both formulas were administered 3 times daily (AM, midday, PM) in four caplets for 1 month (Table 1). Both are described above. The first formula (C-Med-100®) is a patented water extract (U.S. patents 6,039,949; 6,238,675 B1) contract manufactured by Laboratorio Centroflora (Sao Paulo, Brazil) and distributed in North America by AF Nutraceuticals (Morristown, NJ), IHT Healthcare Products (Hillside, NJ), Optigene-x (Morristown, NJ), and Bioceuticals, Inc. (Naples, FL). It is formulated and based on the historical medicinal use of Cat's Claw. It is a water-soluble extract ultra-filtrated to remove high molecular weight toxic conjugates (>10,000 MW), containing 8–10% carboxy alkyl esters (CAE) as active ingredients, and it is essentially free of oxindole alkaloids (<0.05%[15]). The active ingredients of C-Med-100® (85% of them) absorb onto charcoal, have a UV absorption maximum of 200 nm, and react with hydroxylamine and ferric chloride, characterizing them as esters.

Biomarker analyses

Four biomarkers that can independently indicate DNA damage accumulation have been used in this study. They are (i) Interleukins 1a and 1b, which are proinflammatory cytokines produced by inflammatory cells responding to

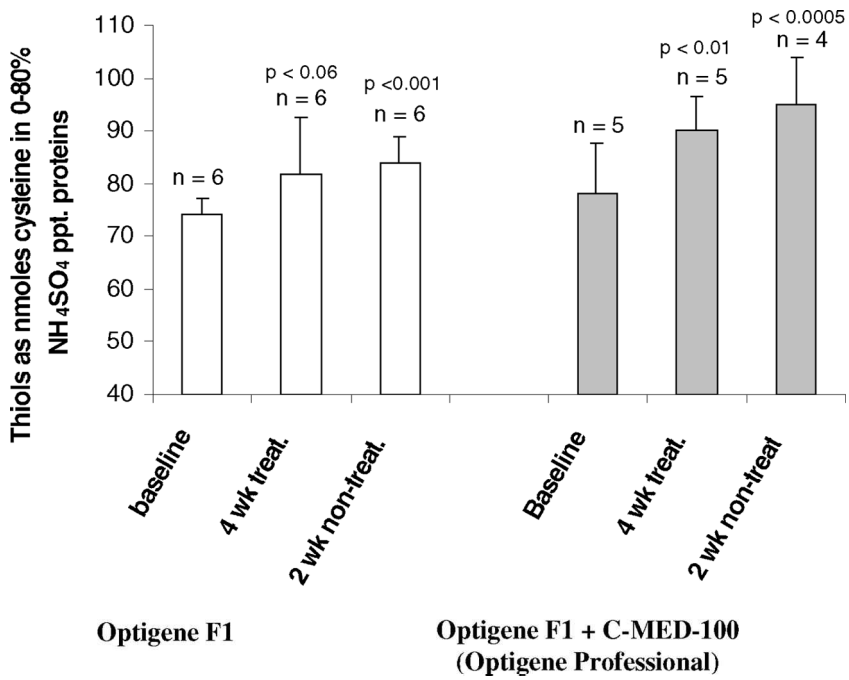

FIG. 2. Redox balance as a surrogate estimate of DNA repair were analyzed in plasma samples as nmoles cysteine in 0–80% ammonium sulfate precipitated protein before (baseline), after 4 weeks of supplement (treatment), and after 2 weeks more at washout (non-treatment). Mean SD shown and comparisons made by paired *t* test to before-treatment values.

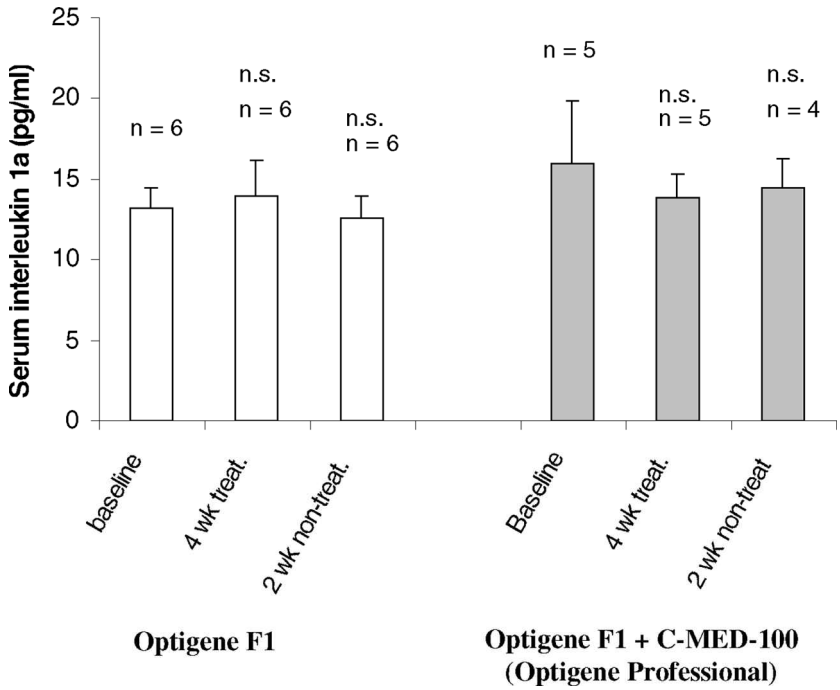

FIG. 3. Interleukin 1a was determined in plasma samples as pg/mL before (baseline), after 4 weeks of supplement (treatment), and after 2 weeks more of washout (non-treatment). Mean SD shown and comparisons made by paired *t* test to before-treatment values.

oxidative stress signals, (ii) (8-OH) guanine adducts in lymphocyte DNA, and (iii) plasma/serum protein thiols, which are a surrogate indicator of endogenous oxidative stress production by estimating the conversion of thiols to disulfides, which in turn can indicate critical DNA repair dysfunction such as with PARP (poly ADP-ribose polymerase).

Interleukins 1a and 1b analyses

Interleukins 1alpha (1a) and 1beta (1b) were measured by solid phase sandwich enzyme-linked Immunosorbent assay using kits manufactured by Bioscience International, Inc. (Camarillo, CA).

(8-OH) adducts

This biomarker was determined in DNA isolated from peripheral blood lymphocytes by addition to the cell pellet of 250 μl of homogenizing buffer (pH = 7.3) containing 0.3 M sucrose, 0.025 M Tris, and 0.002 M EDTA. The rest of the procedure is described in detail elsewhere.[17]

Plasma thiols

The thiol tests were performed on 200 μl aliquots of serum that had been precipitated with 80% saturated ammonium sulfate as previously described in detail elsewhere[18]. Briefly, the precipitated pellets were harvested by centrifugation, dissolved in physiologic saline, the DTNB (5,5′-dithio-bis(2-nitrobenzoic acid) reagent added and the absorbance at 412 nanometers read against appropriate blanks as: (sample + DTNB) − (sample + saline) − DTNB + saline). The thiols present in 80% saturated ammonium sulfate precipitated serum were quantified as nM cysteine residues in the precipitated protein fraction per 0.2 ml serum. Serum samples were analyzed in duplicate.

Statistical analyses

The analyses were performed as paired *t* test comparisons of sample group means using SPSS software package (SPSS, Inc.) before, after 4 weeks at supplement and after 2 more weeks of washout of subjects receiving either formula. One patient in the 2-week washout group was a laboratory failure and lost sample.

Hence, when comparing the 2-week washout group with the before values, only four paired samples could be used.

RESULTS

General health of study population

No apparent acute or chronic diseases were reported by either the subjects or the attending physician before or during this study. There were also no side effects or symptoms observed or recorded that could be attributed to either supplement or to the washout period for the subjects being evaluated. Physical examination and interviews conducted by the attending physician also revealed no apparent medical changes in signs or symptoms.

(8-OH) guanine DNA adducts

The direct measure of DNA damage in peripheral lymphocytes was used in this study as the benchmark biochemical test to evaluate the efficacy of anti-aging nutritional therapy, which was in turn designed to reduce the genetic hazards of oxidant-generated radicals by administration of supplement containing antioxidants. The data reported in Figure 1 demonstrate that both formulas were very effective at reducing DNA damage in lymphocytes exposed daily *in vivo* to the supplements for 4 weeks. Even after 2 weeks of washout following the 4-week supplementation period, there still remained statistically significant effects with the second supplement. The more persistent reduction in DNA damage could be directly attributed to the presence of C-Med-100 in the second formula, since the first formula contains all ingredients of second formula except C-Med-100 (Table 1), and the (8-OH) adducts/10^9 nucleotide bases in DNA after use of the second formula remained significantly reduced even after 2 weeks of washout (Fig. 1).

Plasma thiols as a surrogate DNA repair indicator

Plasma/serum thiols are a good estimate of endogenous oxidative stress *in vivo* as they reflect reduction/oxidation (redox) balance throughout the body due to peripheral circulation (i.e., exposure) of blood to all tissues. Because amino acids such as cysteine can easily

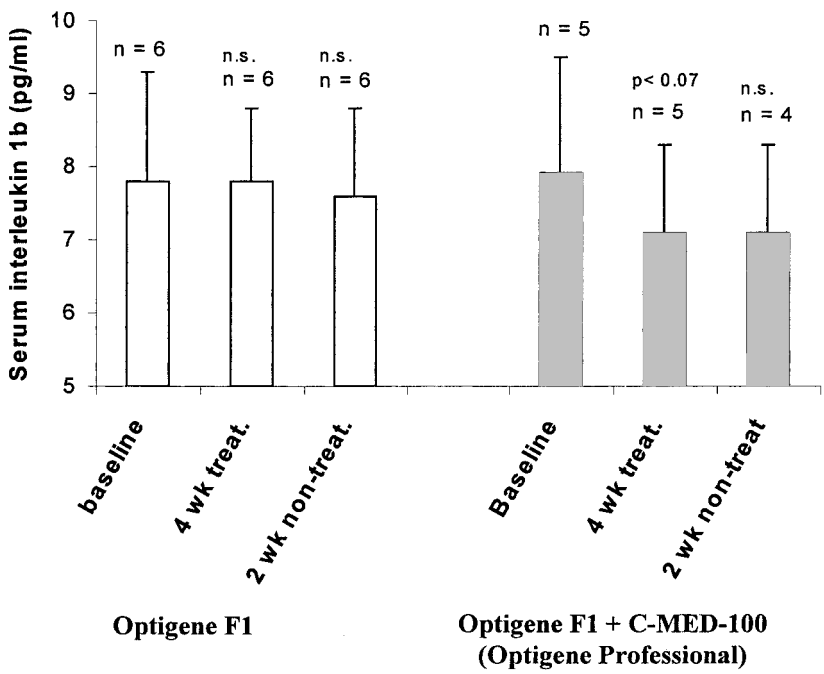

FIG. 4. Interleukin 1b was determined in plasma samples as pg/mL before (baseline), after 4 weeks at supplement (treatment), and after 2 weeks more of washout (non-treatment). Mean SD shown and comparisons made by paired *t* test to before-treatment values.

react with oxidative radicals converting thiols to disulfides, the relative balance of thiols to disulfide forms can be estimated colormetrically to indicate the redox balance in serum. The estimates made for this study have been carried out on 0–80% ammonium sulfate precipitated proteins in plasma, in order to avoid simple modulation from dietary components such as cysteine, uric acid, vitamin C, carotenoids, and glutathione, and thus allow the focus on redox balance measurement to be concentrated on potential signal transducting proteins in plasma. Thiol status of serum/plasma proteins have already been shown to estimate PARP (DNA repair) activity and longevity of mammals.[8,18] Therefore, plasma thiols surrogately estimate DNA repair capacity and, consequently, the potential level of DNA damage remaining.

The data comparing the two formulas are presented in Figure 2. It can easily be seen that both formulas administered for four weeks were associated with a concomitant elevation in plasma thiol status, clearly indicating that these dietary interventions were successful in reducing the endogenous oxidative stress levels of the supplemented subjects. This result is strongly supported by the (8-OH) adduct data presented in Figure 1, because increased DNA repair reflected by increased thiol status in plasma would predict less DNA damage. Moreover, both the magnitude of the thiol increase and the significance level supported that the second formula (containing C-Med-100) was more effective at enhancing DNA repair and thereby resisting DNA damage accumulation.

Interleukins 1a and 1b

The serum level of pro-inflammatory cytokines are strong indicators of endogenous oxidative stress, because activated phagocytic cells initiate an inflammatory response by producing both oxygen radicals and inflammatory cytokines. Therefore, we have estimated the serum levels of interleukins 1a and 1b as indicators of oxidative stress leading to DNA damage. Whereas interleukin 1a was not affected by either formula (Fig. 3), interleukin 1b showed a tendency toward reduction by the second formula (4 weeks of treatment + 2 weeks of washout data pooled gave $p < 0.05$; Fig. 4) but not the first formula. Again these data support the results obtained with the (8-OH) adduct- and plasma thiol-biomarkers, which showed that endpoints sensitive to endogenous oxidative stress were all modulated toward reduced DNA damage potential *in vivo* especially by the second formula (containing C-Med-100).

DISCUSSION

DNA damage as the source of cellular mutation and a significant cause of chronic disease is now one of the oldest and best substantiated medical hypotheses.[2,6] Oxidative stress, related to the rate of oxygen consumption, dietary factors, and general exposure, has been identified as the major variable in causing DNA damage.[2,7] Oxidative stress can be genetically inherited (as occurs in familial polyposis and ulcerative colitis) or acquired (as in HIV infection and dietary exposure to oxygen-radicals). Moreover, oxidative stress contributes to a down regulation in DNA repair.[6,19] Together, these data suggest that measures of oxidative stress can serve as indicators of the sorts of physiological damage normally associated with aging.

In this study, two formulas designed to provide strong anti-oxidant dietary protection both significantly reduced DNA and pro-oxidant induced damage as estimated by several independen biomarkers (Figs. 1–4). These data suggest the feasibility of preventing DNA damage and age-related consequences, by administering targeted nutritional support. In addition, C-MED-100 clearly enhances this effect. The reason for the enhanced efficacy is unclear, but likely related to an enhancement of DNA repair, presumably via NF-kB inhibition.[10,11,14–16]

To our knowledge, only one other antioxidant supplement (a combination of carotenoids, nicotinamide and zinc)[13] has shown to improve clinical biomarker values, often felt to be important in putative anti-aging treatments. Together these studies strongly support the efficacy of combining non-competitive antioxidant components into an additive/synergistic formulation for treatment of age-related biomarkers.

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