

## Original Research

# Inadequate Antioxidant Nutrient Intake and Altered Plasma Antioxidant Status of Rheumatoid Arthritis Patients

Sang-Cheol Bae, MD, PhD, MPH, Soo-Jin Kim, MSc, Mi-Kyung Sung, PhD

*Department of Internal Medicine, Division of Rheumatology, Hanyang University College of Medicine and the Hospital for Rheumatic Diseases (S.-C.B.), Department of Food and Nutrition, Sookmyung Women's University (S.-J.K., M.-K.S.), Seoul, KOREA*

**Key words:** rheumatoid arthritis, antioxidants, superoxide dismutase, glutathione peroxidase, malondialdehyde

**Objective:** Elevated free radical generation in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA). The present study was performed to evaluate dietary nutrient intake and plasma oxidant/antioxidant status in RA patients.

**Methods:** RA patients (n = 97) and their age, gender-matched controls (n = 97) participated in this cross-sectional case-control study. Nutrient intake was estimated using a semi-quantitative food frequency questionnaire. Twenty subjects from each group provided blood samples, and plasma concentrations of  $\alpha$ -tocopherol and malondialdehyde (MDA) were measured. Also, plasma activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured.

**Results:** The mean calorie intake of RA patients was lower than that of the healthy controls. Energy-adjusted intake of fat, vitamin A and  $\beta$ -carotene were significantly lower in patients than those of the control subjects. RA patients had a decreased mean plasma  $\alpha$ -tocopherol level. The activity of plasma SOD and GPx in patients was significantly lower than that in control subjects.

**Conclusion:** These results suggest proper antioxidant nutrient intake management may reduce free radical generation and improve antioxidant status in RA patients.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic syndrome of unknown etiology and is characterized by non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues and joint deformities. It affects nearly 1% of the population worldwide [1]. Studies have indicated that the development of RA is partly related to the excess production of reactive oxygen species and a lowered ability to remove oxidative stress [2,3]. A recent study indicated that pro-inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$  are involved in the formation of toxic peroxynitrite by increasing the activity of nitric oxide synthase [4].

Clinical evidence has suggested oxidative stress is elevated in RA patients. Plasma malondialdehyde, a degradation product

of lipid peroxidation, level was significantly higher in the synovial fluid and serum of RA patients than that of control subjects [5,6]. Also, in children with juvenile rheumatoid arthritis, plasma and red blood cell alpha-tocopherol concentrations were lower compared to those of healthy children [7]. In a Finnish cohort study, low alpha-tocopherol status was suggested as a risk factor for RA [8]. RA patients show not only low levels of antioxidants in the blood, but altered activity of blood antioxidant enzymes including glutathione peroxidase (GPx) [2], CuZn superoxide dismutase (SOD) [6,7,9] and catalase [10,11], although study results are not consistent.

Based on previous reports, diets high in major dietary antioxidants such as vitamin E, vitamin C,  $\beta$ -carotene and phenolic compounds have been suggested to alleviate RA symptoms, possibly by reducing disease-related oxidative stress.

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Address reprint requests to: Mi-Kyung Sung, Ph.D., Associate Professor, Department of Food and Nutrition, Sookmyung Women's University, Chungpa-dong 2-ka, Yongsan-ku, Seoul, 140-742, KOREA. E-mail: mksung@sookmyung.ac.kr

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However, few studies have been conducted to evaluate the nutrient intake in RA patients.

The objective of this study was to evaluate dietary intake of major nutrients including antioxidants and measure plasma antioxidant/oxidant status in rheumatoid arthritis patients and their age, gender-matched controls.

## MATERIALS AND METHODS

### Subjects

Rheumatoid arthritis patients ( $n = 97$ ) and age, gender-matched healthy controls ( $n = 97$ ) were recruited into the study. All patients fulfilled the revised American College of Rheumatology (ACR) criteria for RA [12]. Consent for all procedures was obtained from each individual and from the university research ethics committee. Blood samples were taken from subsets of the patients ( $n = 20$ ) and controls ( $n = 20$ ). The patients were chosen for the study after having a preliminary evaluation consisting of a brief medical history, smoking and alcohol habits and physical examinations. Patients with any history of liver diseases, diabetes mellitus, respiratory disorders and cardiovascular diseases were not included in the study.

### Estimation of Nutrient Intake from Food Frequency Questionnaire

We used a interviewer-administered semi-quantitative food frequency questionnaire to estimate nutrient intake of the subjects. The questionnaire included a list of 102 food items. Selection criteria were 1) most frequently consumed food items, 2) food items consumed in greatest amounts and 3) major food items supplying each nutrient, especially antioxidant vitamins. The selection was based on the 1998 National Health and Nutrition Survey Report [13]. Selected food items were categorized according to food groups and subdivided by food preparation methods, nutrient content and portion sizes. Categories and numbers of food items in each category were cereals and starches-17, meats-16, fishes & other seafoods-5, fruits-10, eggs-1, legumes-6, vegetables-22, milk and dairy products-5, oils-2, hot beverages & soft drinks-7 and alcoholic beverages-8. Subjects were asked to state the average frequency of consumption of each food item according to the categories of frequency, none to three times a day. The portion sizes were set as follows: a 1/2 serving size, a serving size, and a 1.5 serving size. The interviewer showed food models and photographs of the standard serving size, and asked the subjects to refer to those portions when selecting the amount of food consumed. The food frequency questionnaire was coded and analysed for nutrient intake by a computer aided nutrient analysis program for professionals (CAN-PRO, APAC Intelligence, Seoul, Korea).

### Blood Sample Analysis

Fasting blood samples were collected in heparin-containing tubes and left at room temperature for one hour. The samples were centrifuged for 15 minutes at 4°C, 220 g. Plasma was stored at -70°C until analysis.  $\alpha$ -Tocopherol was extracted to hexane from plasma and was then quantified by HPLC with a UV detector at 292nm. A Waters MicroBondapak C18 column was used for separation. Plasma malondialdehyde content was determined based on Yagi's method [14]. Briefly, 4 mL of 1/12N H<sub>2</sub>SO<sub>4</sub> and 0.5 mL phosphotungstic acid were added to 100  $\mu$ L of plasma. The mixture was incubated at room temperature for five minutes, and then centrifuged at 220 g for three minutes. The pellet was collected and washed with 2 mL of 1/12N H<sub>2</sub>SO<sub>4</sub> and 0.3 mL phosphotungstic acid at 220 g for three minutes. The pellet was incubated with 2 mL of distilled water and 1 mL of 0.67% thiobarbituric acid solution for one hour. Absorbance was read fluorometrically at Ex 515 nm and Em 553 nm. Plasma SOD activity was measured based on the method of Flohe *et al.* [15]. Five  $\mu$ mol of xanthine was added to 50 mM phosphate buffer solution to prepare cytochrome C solution. Two mL of cytochrome C solution and 50  $\mu$ L of 0.2 unit xanthine oxidase were mixed with an aliquot of plasma and the amount of reduced xanthine was determined at 550 nm. One unit of SOD corresponds to 50% inhibition of cytochrome C reduction. Plasma GPx activity was measured based on the method Flohe *et al.* [16]. An aliquot of plasma was mixed with the reaction mixture (0.1 M potassium phosphate buffer containing 1 mM EDTA, 2.4U/mL glutathione reductase, 10 mM glutathione) and incubated for 10 minutes at 37°C. NADPH and 12 mM t-butyl hydroperoxide were added to the reaction mixture, and the amount of reduced glutathione was determined at 340 nm for three minutes.

### Statistical Analysis

The results are given as mean  $\pm$  SEM values. The significance of the mean difference between groups was assessed by the Student's *t* test.

## RESULTS

### Demographic Characteristics

Table 1 shows the demographic characteristics of RA patients and their age-gender matched controls. The mean height, monthly income and smoking status of the RA patients were not significantly different from those of their controls. However, the mean weight and body mass index were significantly lower, and the mean education period was shorter in the patient group.

### Nutrient Intake of Study Subjects

The daily nutrient intake is shown in Table 2. The mean energy intake of the RA patients was slightly lower than that of

**Table 1.** Demographic Characteristics of Patients with RA and their Age-Gender Matched Controls

	RA (n = 97)	Control (n = 97)	p value
Age (years)	56.4 ± 1.7 <sup>1</sup>	55.2 ± 1.2	N.S. <sup>2</sup>
Gender			N.S.
Female	92 (93%) <sup>3</sup>	92 (93%)	
Male	7 (7%)	7 (7%)	
Height	157.9 ± 0.7	158.5 ± 0.6	N.S.
Weight	53.8 ± 0.9	57.8 ± 0.8	<0.01
BMI (kg/m <sup>2</sup> )	21.5 ± 0.3	23.0 ± 0.3	<0.01
Education (years)			<0.05
≤12	84%	68%	
>12	16%	32%	
Income (US\$/year)			N.S.
<10,000	23%	22%	
10,000–20,000	30%	28%	
20,000–30,000	21%	19%	
30,000–40,000	9%	13%	
>40,000	7%	17%	
Smoking			N.S.
Smoker	10%	4%	
Ex Smoker	5%	4%	
Non Smoker	85%	92%	

<sup>1</sup> Values are mean ± SEM.

<sup>2</sup> Not significant.

<sup>3</sup> (no. of subjects/total no. of subjects in the group) × 100.

**Table 2.** Daily Intake of Energy and Nutrient Assessed by the Semi-Quantitative Food Frequency Questionnaire

	Group		p value
	RA (n = 97)	Control (n = 97)	
Calorie (kcal)	1838.35 ± 47.62 <sup>1</sup>	1933.89 ± 43.36	N.S. <sup>2</sup>
Protein (g)	69.83 ± 2.40	77.18 ± 2.50	<0.05
Fat (g)	43.67 ± 1.88	50.30 ± 2.06	<0.05
Carbohydrate (g)	289.65 ± 6.97	292.33 ± 5.60	N.S.
Ca (mg)	625.41 ± 24.00	663.92 ± 25.39	N.S.
P (mg)	1163.07 ± 35.38	1249.04 ± 36.00	N.S.
Fe (mg)	11.16 ± 0.37	12.43 ± 0.42	<0.05
Total Vit A (μg RE)	682.55 ± 35.77	843.73 ± 45.62	<0.001
Retinol (μg)	105.75 ± 7.48	123.43 ± 10.15	N.S.
β-Carotene (μg)	2869.04 ± 149.33	3736.83 ± 226.62	<0.01
Vit B <sub>1</sub> (mg)	1.30 ± 0.04	1.36 ± 0.04	N.S.
Vit B <sub>2</sub> (mg)	1.21 ± 0.05	1.35 ± 0.05	<0.05
Niacin (mg)	15.81 ± 0.57	17.97 ± 0.64	<0.05
Vit C (mg)	125.98 ± 5.86	139.53 ± 6.72	N.S.
Cholesterol (mg)	180.28 ± 14.82	193.23 ± 9.88	N.S.

<sup>1</sup> Values are mean ± SEM.

<sup>2</sup> Not significant.

the control subjects. Also, RA patients consume less protein, fat, iron, total vitamin A, β-carotene, vitamin B<sub>2</sub> and niacin than control subjects do. Since the patients had less calorie intake, the mean intake of each nutrient was adjusted for calorie consumption to compare net nutrient intake between the two groups (Table 3). Results indicate that intake of fat, carbohydrate, vitamin A and β-carotene per 1,000 kcal in RA patients was significantly lower compared to that of the control subjects. The mean intake of vitamin A and β-carotene, major

**Table 3.** Daily Nutrients Intake per 1000 kcal Calorie Intake Assessed by the Semi-Quantitative Food Frequency Questionnaire

	Group		p value
	RA (n = 97)	Control (n = 97)	
Calorie (kcal)	1000	1000	N.S. <sup>2</sup>
Protein (g)	37.86 ± 0.67 <sup>1</sup>	39.33 ± 0.57	N.S.
Fat (g)	23.44 ± 0.56	25.30 ± 0.61	<0.05
Carbohydrate (g)	158.44 ± 1.92	152.96 ± 1.84	<0.05
Ca (mg)	344.38 ± 10.34	340.79 ± 9.04	N.S.
P (mg)	636.59 ± 10.71	641.24 ± 7.89	N.S.
Fe (mg)	6.11 ± 0.13	6.36 ± 0.12	N.S.
Total Vit (μg RE)	370.86 ± 17.72	423.85 ± 16.25	<0.05
Retinol (μg)	55.60 ± 3.16	61.93 ± 4.58	N.S.
β-Carotene (μg)	1567.70 ± 80.11	1868.14 ± 83.06	<0.01
Vit B <sub>1</sub> (mg)	0.72 ± 0.01	0.70 ± 0.01	N.S.
Vit B <sub>2</sub> (mg)	0.66 ± 0.02	0.68 ± 0.02	N.S.
Niacin (mg)	8.63 ± 0.22	9.12 ± 0.17	N.S.
Vit C (mg)	69.97 ± 3.01	70.91 ± 2.51	N.S.
Cholesterol (mg)	93.99 ± 5.5	97.78 ± 3.89	N.S.

<sup>1</sup> Values are mean ± SEM.

<sup>2</sup> Not significant.

antioxidant nutrients, in the patients was 87.4% and 83.9% of the intake in control subjects, respectively. The energy-adjusted mean intake of retinol was lower in RA patients than that of control subjects; however, no statistical difference was found.

### Plasma Indices for Oxidative Stress

Patients with RA had a significantly lower mean plasma α-tocopherol level than the controls (Table 4). Plasma MDA concentration was slightly higher in RA patients than that of control subjects; however, no statistical significance was found. Plasma SOD and GPx activities were significantly lower in RA patients, being 62% and 80% of those in control subjects.

## DISCUSSION

The present study was performed to evaluate nutrient intake especially of antioxidants in patients with RA and to assess

**Table 4.** Plasma Parameters of Oxidant/Antioxidant Status in RA Patients and Healthy Controls

	Group		p value
	RA	Control	
α-Tocopherol (mg/L)	10.07 ± 0.75 <sup>1</sup>	13.88 ± 0.66	<0.001
Malondialdehyde (nmol/mL)	3.02 ± 0.14	2.82 ± 0.10	N.S. <sup>2</sup>
Superoxide Dismutase (unit/mL)	32.53 ± 2.44	51.77 ± 2.85	<0.001
Glutathione Peroxidase (unit/mL)	0.033 ± 0.002	0.041 ± 0.002	<0.05

<sup>1</sup> Values are mean ± SEM.

<sup>2</sup> Not significant.

oxidative stress markers in the blood. Recent investigations have consistently indicated that the inflammatory process produces oxygen radicals and decreased antioxidant levels, which may worsen the symptoms of the rheumatoid arthritis [4,17]. However, few studies have been conducted to examine antioxidant nutrient intake of patients.

Results from this study indicate that daily intake of total vitamin A and  $\beta$ -carotene was significantly lower in the patients compared to that of the controls. The consumption of major macronutrients and total calories was also lower in patients although only fat intake showed a significance after calorie adjustment was made. Roubenoff *et al.* [18] showed protein-energy malnutrition (PEM) among RA patients. The increased production of cytokines is known to induce anorexia in cancer patients [19]. Therefore, the increased production of inflammatory cytokines may be a possible cause of PEM in RA patients.

A number of studies have indicated that the blood markers of antioxidant nutrient status in RA patients are significantly lower than those of controls. To the present, the decreased antioxidant status of RA patients has been explained by excessive need for antioxidants due to the inflammatory process itself. However, results from this study imply that the decreased antioxidant nutrient intake of RA patients is another possible contributing factor to decreased antioxidant status. A study conducted by Morgan *et al.* [20] also indicated that the antioxidant nutrient intake and plasma levels are not optimal in RA patients. Stone *et al.* [21] reported calcium, folic acid, vitamin E, zinc and selenium intake did not meet RDI in an observational study of forty-eight patients. However, nutrient intake of juvenile arthritis patients was not different from that of their healthy counterparts [22].

As previous investigations indicated, the present study showed that plasma markers of antioxidant status in RA patients are poor. Although definite evidence for the cause-effect of antioxidant levels in RA is not available, Araujo *et al.* [23] implied a decreased level of vitamin E is a possible cause of disease development. Also, serum concentrations of  $\alpha$ -tocopherol, retinal and  $\beta$ -carotene were suggested as possible risk factors for developing RA in a 15-year follow-up study conducted by Comstock *et al.* [24]. Edmonds *et al.* [25] showed vitamin E supplementation (600 mg/day) improved clinical symptoms of RA patients. A possible mechanism by which vitamin E alleviated RA symptoms is reduced formation of prostaglandins, major molecules produced during the inflammation process [26].

Results from this study also showed significantly decreased activity of SOD and GPx in RA patients compared to that of the controls, and this is in agreement with previous reports [2,3,9]. DiSilvestro *et al.* [27] showed that the administration of anti-inflammatory drugs increases plasma SOD activity, indicating the inflammation process produces free radicals, thereby decreasing SOD activity. Disease itself may inhibit the activity of SOD and reduce the synthesis of SOD [28]. Tarp *et al.* [29]

showed that the concentration of blood selenium, a component of GPx, was lower in RA patients than that of healthy subjects, and selenium supplementation increased blood GPx activity in RA patients. Also, Thabrew *et al.* [30] indicated increases in serum SOD and GPx activity in RA patients treated with antioxidant herbal preparations resulted either from transcriptional activation of these enzymes or removal of oxidative stress. Helmy *et al.* [17] showed that the combination of standard treatment and antioxidants increases serum GPx activity with better disease control, including morning stiffness. These results indicate improvement in antioxidant status of RA patients may ease disease symptoms.

It is not possible to conclude from this study that the decreased levels of plasma antioxidants and the decreased activity of antioxidant enzymes are due to either the lower antioxidant nutrient intake or the active inflammatory disease itself. Mechanistic studies on the relationship among oxidative stress, antioxidant defense and RA development will give better insights into a cause-effect relationship. Also, a large-scale cohort study is required to define the role of antioxidants in RA management. Nevertheless, this study indicates that proper antioxidant nutrient intake management may be important in alleviating RA symptoms.

## REFERENCES

1. Harria, ED: Etiology and pathogenesis of rheumatoid arthritis. In Kelly WN, Harris ED, Ruddy S, Sledge DB (eds): "Textbook of Rheumatology." Philadelphia: WB Saunders, pp. 833–873, 1993.
2. Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ: The glutathione defense system in the pathogenesis of rheumatoid arthritis. *J Appl Toxicol* 21:69–73, 2001.
3. Çimen MYB, Çimen ÖB, Kaçmaz M, Öztürk JS, Yorgancıoğlu and Durak İI: Oxidant/antioxidant status of the erythrocytes from patients with rheumatoid arthritis. *Clin Rheumatol* 19:275–277, 2000.
4. Darlington LG, Stone TW: Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. *Br J Nutr* 85:251–269, 2001.
5. Gambhir JK, Lali P, Jain AK: Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. *Clin Biochem* 30:351–155, 1997.
6. Kiziltunç A, Çoğalgil Ş, and Cerrahoğlu L: Carnithine and antioxidants levels in patients with rheumatoid arthritis. *Scand J Rheumatol* 27:441–445, 1998.
7. Sklodowska M, Gromadzińska J, Biernacka M, Wasowicz W, Wolkanin P, Marszalek A, Brózik H, Pokuszyńska K: Vitamin E, thiobarbituric acid reactive substance concentrations and superoxide dismutase activity in the blood of children with juvenile rheumatoid arthritis. *Clin Exp Rheumatol* 14:433–439, 1996.
8. Knekt P, Heliövaara M, Aho K, Alfthan G, Marniemi J, Aromaa A: Serum selenium, serum alpha-tocopherol, and the risk of rheumatoid arthritis. *Epidemiol*, 11:402–105, 2000.
9. Taraza C, Mohora M, Vargolici B, Dinu V: Importance of reactive oxygen species in rheumatoid arthritis. *Rom J Intern Med*, 35:89–98, 1997.

10. Kerimova AA, Atalay M, Yusifov EY, Kuprin SP, Kerimov TM: Antioxidant enzymes; possible mechanism of gold compound treatment in rheumatoid arthritis. *Pathophysiol* 7:209–213, 2000.
11. Kiziltunc A, Cogalgil S, Cerrahoglu L: Carnitine and antioxidants levels in patients with rheumatoid arthritis. *Scand J Rheumatol* 27:441–445, 1998.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheuma* 31: 315–324, 1988.
13. The Ministry of Health and Welfare Korea: Report on 1998 National Health and Nutrition Survey, 1999.
14. Yagi K: A simple fluorometric assay for lipid peroxides in blood serum or plasma. In Miquel J, Quintanilha AT, Weber H (eds): "CRC Handbook of Free Radicals and Antioxidants in Biomedicine." Boca Raton, FL: CRC Press, pp 215–217, 1988.
15. Flohé L, Becker R, Brigelius R, Lengfelder E, Ötting F: Convenient assay for superoxide dismutase. In Miquel J, Quintanilha AT, Weber H (eds): "CRC Handbook of Free Radicals and Antioxidants in Biomedicine." Boca Raton, FL: CRC Press, pp 287–288, 1988.
16. Flohe L, Gunzler WA: Assays of glutathione peroxidase. *Method Enzymol* 105:114–121, 1984.
17. Helmy M, Shohayeb M, Helmy MH, El-Bassiouni EA: Antioxidants as adjuvant therapy in rheumatoid disease. *Arzneim-Forsch/ Drug Res* 51:293–298, 2001.
18. Roubenoff R, Roubenoff RA, Ward LM, Holland SM, Hellmann DB: Rheumatoid cachexia: depletion of lean body mass in rheumatoid arthritis. Possible association with tumor necrosis factor. *J Rheumatol* 19:1505–1510, 1992.
19. Mason JB, Choi S-W: Nutritional assessment and management of the cancer patients. In Bronner F (ed): "Nutritional Aspects of Clinical Management of Chronic Disorders and Diseases," Boca Raton, FL: CRC Press, pp 201–204, 2002.
20. Morgan SL, Anderson AM, Hood SM, Matthews PA, Lee JY, Alarcon GS: Nutrient intake patterns, body mass index, and vitamin levels in patients with rheumatoid arthritis. *Arthritis Care Res* 10:9–17, 1997.
21. Stone J, Doube A, Dudson D, Wallace J: Inadequate calcium, folic acid, vitamin E, zinc, and selenium intake in rheumatoid arthritis patients: results of a dietary survey. *Semin Arthritis Rheum* 27: 180–185, 1997.
22. Helgeland M, Svendsen E, Forre O, Haugen M: Dietary intake and serum concentrations of antioxidants in children with juvenile arthritis. *Clin Exp Rheumatol* 18:637–641, 2000.
23. Araujo V, Arnal C, Boronat M, Ruiz E, Dominguez C: Oxidant-antioxidant imbalance in blood of children with juvenile rheumatoid arthritis. *Biofactors* 8:155–159, 1998.
24. Comstock GW, Burke AE, Hoffman SC, Heizlsouer KJ, Bendich A, Masi AT, Norkus EP, Malamet RL, Gershwin ME: Serum concentrations of alpha tocopherol, beta carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* 56:323–325, 1997.
25. Edmonds SE, Winyard PG, Guo R, Kidd B, Merry P, Lnagrish-Smith A, Hansen C, Ramm S, Blake DR: Putative analgesic activity of repeated oral doses of vitamin E in the treatment of rheumatoid arthritis. Results of a prospective placebo controlled double blind trial. *Ann Rheum Dis* 56:649–655, 1997.
26. Halevy O, Sklan D: Inhibition of arachidonic acid oxidation by beta-carotene, retinal and alpha-tocopherol. *Biochim Biophys Acta* 918:304–307, 1987.
27. DiSilvestro RA, Marten J, Skehan M: Effects of copper supplementation on ceruloplasmin and Cu/Zn superoxide dismutase in free-living rheumatoid arthritis patients. *J Am Coll Nutr* 11:177–180, 1992.
28. Puscas I, Coltau M, Baican M, Domuta G: Omeprazole has a dual mechanism of action: it inhibits both H(+)/K(+)ATPase and gastric mucosa carbonic anhydrase enzyme in humans (in vitro and in vivo experiments). *J Pharmacol Exp Ther* 290:530–534, 1999.
29. Tarp U, Hansen JC, Overvad K, Thorling EB, Tarp BD, Graudal H: Glutathione peroxidase activity in patients with rheumatoid arthritis and in normal subject: effect of long-term selenium supplementation. *Arthritis Rheum* 30:1162–1166, 1987.
30. Thabrew MI, Senaratna L, Samarawickrema N, Munasinghe C: Antioxidant potential of two polyherbal preparations used in Ayurveda for the treatment of rheumatoid arthritis. *J Ethnopharmacol* 76:285–291, 2001.

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