



from the desk of YIBING WANG, M.D., Ph.D.

.....
VEMMA DIRECTOR OF PRODUCT DEVELOPMENT AND RESEARCH

Valued Vemma Brand Partner,

It's a great honor to have the Vemma immunity study and Vemma bioavailability study accepted for publication in two reputable journals such as the *Journal of Medicinal Food* and *Journal of Agricultural and Food Chemistry*[®] respectively. Needless to say, we are thrilled to share this impressive news.

The studies you are about to read give credence to the countless positive testimonials Vemma has received from customers over the years on its ability to help overcome challenges, increase vitality and enhance well-being.*

The first study, led by Dr. Boxin Ou from Brunswick Laboratories in Massachusetts, entitled "Effect of a Mangosteen Dietary Supplement on Human Immune Function: A Randomized, Double-Blind, Placebo-Controlled Trial," examined C-reactive protein response and immune-regulatory response in the human body. This trial, which took place over the course of 30 days, involved 29 men and 30 women ages 40 to 60. Subjects were randomly divided into two groups, the Vemma formula or a placebo, with about the same number of male and female participants in each group.

After baseline blood tests were completed, participants received a dose (2 ounces/59 ml) of either the Vemma formula or an identical inactive placebo each morning before breakfast. Blood samples were collected from each subject on day zero and after 30 days of taking the product. The researchers were looking for changes in the blood relating to C-reactive protein and immunity. C-reactive protein is an important blood marker for optimal health, the higher the level, the higher the risk for health challenges. Immunity markers in the blood can show how well the body is able to fight health challenges.

Through blood analysis researchers identified markers that enabled them to conclude that the consumption of the Vemma formula resulted in a significant decrease in C-reactive protein and a significant increase in immune system function.*

The icing on the cake, so to speak, was how participants responded to a survey at the end of the trial. All of the participants who had taken Vemma reported favorable results, most saying they felt a lot healthier.** The level of satisfaction was significantly lower in the placebo group.

The data and positive feedback obtained from this study confirms that consuming Vemma daily strengthens the body's natural immune defenses, which can lead people to help overcome health challenges, maintain their vitality and enhance overall quality of life.*

The second study, also led by Dr. Boxin Ou from Brunswick Laboratories, entitled "Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (*Garcinia mangostana*) Product in Humans", was designed to determine the overall bioavailability (proportion which is absorbed and utilized by the body) and antioxidant effects of Vemma's formula in the human body. This trial, which took place over a 24 hour period of time, involved an equal number of generally healthy male and female subjects between 20 and 23 years of age. Subjects were randomly divided into two groups, placebo and the Vemma formula.

.....
* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

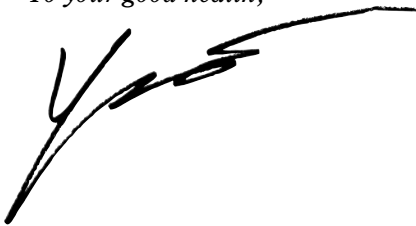
** Results not typical. You may not do as well.

Before the products were consumed, each participant's blood was measured. After the blood tests were completed, participants received either a single dose (2 ounces/59 mL) of the Vemma formula or an identical inactive placebo before breakfast. Blood samples were collected from each participant before and numerous times after taking the Vemma formula or placebo to track the presence of nutrients that would show a change in the antioxidant status of the blood. Antioxidants are known to scavenge free radicals (unstable oxygen molecules that can cause damage in the body) and thus help to prevent serious health challenges.

The noteworthy nutrients that showed up in the blood serum of the Vemma participants were Vitamins B2, B5, ORAC (antioxidant levels) and alpha-mangostin, each reaching their maximum concentrations. ORAC materialized in the blood within one hour and maintained elevated saturation levels for six hours after intake. In the placebo group, no change in blood serum was observed. Based on these results, the researchers concluded that the Vemma product is highly bioavailable, consequently helping to wage war against serious health challenges.*

In conclusion, the results of these two trials coupled with the overwhelming number of positive testimonies give the Vemma formula a powerful competitive edge.

To your good health,

A handwritten signature in black ink, appearing to read 'Yibing Wang', with a long, sweeping horizontal stroke extending to the right.

Yibing Wang, M.D., Ph.D.
Vemma Director of Product Development and Research

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Note: Regulatory agencies do not require nutritional supplement companies to conduct clinical trials on their products. Because Vemma is confident in its flagship Vemma formula, we elected to fund two clinical trials, conducted by the renowned 3rd party, independent lab, Brunswick Laboratories.



JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

For access to the Vemma
bioavailability study, please go to:

<http://pubs.acs.org/doi/pdfplus/10.1021/jf901012f>

Journal of MEDICINAL FOOD

EDITORS-IN-CHIEF SHELDON S. HENDLER AND MYUNG-SOOK CHOI

KFN Official Journal of the Korean Society of Food Science and Nutrition



Mary Ann Liebert, Inc.  publishers

Effect of a Mangosteen Dietary Supplement on Human Immune Function: A Randomized, Double-Blind, Placebo-Controlled Trial

Yu-Ping Tang,¹ Peng-Gao Li,² Miwako Kondo,³ Hong-Ping Ji,⁴ Yan Kou,⁴ and Boxin Ou³

¹Department of Preventive Medicine, Yanjing Medical School; ²Department of Nutrition and Food Hygiene, School of Public Health and Family Medicine, Capital Medical University, Beijing; ⁴Brunswick Laboratories (China), Suzhou, Jiangsu, China; and ³Brunswick Laboratories, Norton, Massachusetts, USA

ABSTRACT The effect of a mangosteen product containing multivitamins and essential minerals was tested on immune function and well-being in healthy adults. A randomized, double blinded, placebo-controlled study was conducted in 59 healthy human subjects (40–60 years old). Changes from baseline immune function were measured after a 30-day consumption of the mangosteen product and the placebo. The subjects' self-appraisal of their health status was also surveyed. A xanthone-rich mangosteen product intake increased mean values for peripheral T-helper cell frequency ($P = .020$) and reduced the serum C-reactive protein concentration ($P = .014$). Increases in peripheral CD4/CD8 double-positive (DP) T-cell frequency and serum complement C3, C4, and interleukin (IL)-1 α concentrations were significantly higher in the experimental group than in the placebo group (DP, $P = .038$; C3, $P = .017$; C4, $P = .031$; IL-1 α , $P = .006$). At the end of study, serum IL-1 α and IL-1 β concentrations in the study group were significantly higher than that in the placebo group (IL-1 α , $P = .033$; IL-1 β , $P = .04$). Furthermore, more participants in the experimental group reported greatly improved overall health status compared with participants receiving placebo ($P = .001$). The results indicated that the intake of an antioxidant-rich product significantly enhanced immune responses and improved the subject's self-appraisal on his or her overall health status.

KEY WORDS: • *aloe vera* • *C-reactive protein* • *green tea* • *mangosteen* • *peripheral T-lymphocytes* • *T-helper cell* • *xanthone*

INTRODUCTION

THE IMMUNE SYSTEM UNDERGOES persistent and unremitting attack throughout the course of an individual's life, and its function declines with age, especially in the cell-mediated branch.^{1–3} Once the homeostasis within is weakened or functioning improperly, a dysregulation of the immune system occurs. This results in abnormalities in immune responsiveness and vulnerability to infectious disease and cancer, or causing other forms of morbidity and mortality.^{4,5}

Among the numerous factors that exert unfavorable effects on the immune balance, mild malnutrition and excessive oxidative stress within the body are of special importance because they tend to occur frequently. These factors would elevate morbidity and mortality in various conditions and could decrease the quality of life.^{6–11} In this regard, a number of studies have indicated that intake of multivitamins, minerals, and antioxidant supplements may exert beneficial effects on immune function, improving the

quality of life in the elderly and patients with infectious diseases.^{12–17} However, controversies relating to the use of supplements still exist^{18–20} to a certain extent because of the lack of well-designed clinical trials to assess the reputed benefits in humans. Moreover, most studies in the past have focused on people 60 years or older who had various diseases.^{12–20} There are limited numbers of clinical trials on healthy adults ranging from 40 to 60 years of age. However, this age range is a key period for both men and women. During this period, they are likely to face immense social and physiological pressures that may increase the production of free radicals. Poor nutritional status and oxidative stress may lead a decline in immune function and cause morbidity and mortality in later life.²¹

Xanthones, which have potent antioxidative, anti-inflammatory, antibacterial, and various other bioactivities,^{22–31} are rich in a tropical fruit tree, mangosteen, *Garcinia mangostana* L. In the present study, we determine the effect of a xanthone-rich mangosteen juice, enriched with 12 vitamins, selenium, green tea catechins, and aloe vera polysaccharides, on T-cell subsets, cytokine production, and biomarkers of humoral immune responses as well as C-reactive protein (CRP) in healthy adults 40–65 years old.

Manuscript received 5 August 2008. Revision accepted 12 December 2008.

Address correspondence to: Boxin Ou, Ph.D., Brunswick Laboratories, 50 Commerce Way, Norton, MA 02766, USA, E-mail: bou@brunswicklabs.com

SUBJECTS AND METHODS

Study participants

Healthy adult volunteers were recruited into the study. The criteria for participation in the study included the following: between 40 and 60 years of age, free from any chronic illness or serious health problems, no alcohol or drug dependence, no history of organ transplantation, no surgery within the last 12 months, no medication within the last 2 weeks, no intake of supplements or Chinese medicines, and no regular consumption of mangosteen, aloe vera, green tea or other fruits (or its juice). Recruited volunteers were screened by evaluation of medical history, physical examination, and assessment of individuals' habitual life and dietary behaviors using a semiquantitative questionnaire. Sixty subjects (30 women, 30 men) were selected and assigned randomly to the study and the placebo groups. Each group had an equal number of males and females. Fifty-nine volunteers completed the study and were included in the analysis; one man in the placebo group was withdrawn from the study (Fig. 1).

Written informed consent was obtained from each volunteer who participated in the trial. The study was approved by the ethics committee of the Capital Medical University (Beijing, China).

Study design

The study was conducted as a randomized, double blind, placebo-controlled trial. Eligible participants were assigned to the treatment or the placebo groups by permuted randomization. After a 7-day "wash period" and overnight fasting, participants underwent a 30-day study period. Subjects were given either a 59-mL dose per day of a commercially available mangosteen product, Mangosteen Plus™ with Essential Minerals® (Vemma® Nutrition Co., Scottsdale, AZ), or a matching 59-mL dose per day of fructose liquid (Vemma Nutrition Co.). Table 1 lists the

TABLE 1. ENERGY, NUTRIENT, MINERAL, AND SPECIFIC COMPONENT INTAKE WITH THE MANGOSTEEN PRODUCT (59 mL) AND THE PLACEBO LIQUID (59 mL)

| Content per dose | Mangosteen | Placebo |
|---|------------|---------|
| Energy (kcal) | 35 | 35 |
| Total carbohydrate (g) | 8 | 8 |
| Vitamin A (100% as β -carotene) (IU) | 5,000 | BLD |
| Vitamin C (as ascorbic acid) (mg) | 300 | BLD |
| Vitamin D ₃ (as cholecalciferol) (IU) | 1,000 | BLD |
| Vitamin E (as <i>d</i> - α -tocopheryl acetate) (IU) | 60 | BLD |
| Thiamin (as thiamine hydrochloride) (mg) | 1.5 | BLD |
| Riboflavin (as riboflavin USP) (mg) | 1.7 | BLD |
| Niacin (as niacinamide) (mg) | 20 | BLD |
| Vitamin B ₆ (as pyridoxine hydrochloride) (mg) | 5 | BLD |
| Folate (as folic acid) (μ g) | 800 | BLD |
| Vitamin B ₁₂ (as cyanocobalamin) (μ g) | 15 | BLD |
| Biotin (as <i>d</i> -biotin) (μ g) | 300 | BLD |
| Pantothenic acid (as calcium <i>d</i> -pantothenate) (mg) | 10 | BLD |
| Selenium (as amino acid chelate) (μ g) | 140 | BLD |
| Mangosteen/whole leaf aloe vera/green tea blend (g) | 25.2 | BLD |
| Xanthones | | |
| β -Mangostin (mg) | 64.66 | BLD |
| Other mangostins (mg) | 29.45 | BLD |
| Catechins (mg) | 119 | BLD |
| Epigallocatechin gallate (mg) | 3.63 | BLD |
| Plant-sourced mineral blend (mg) ^a | 956 | BLD |

Energy and nutrient composition are data provided by the manufacturer (Vemma Nutrition Co.). BLD, below limit of detection.

^aIncludes a full spectrum of minerals.

ingredients of each liquid. Participants were instructed to drink the liquid before breakfast (usually between 6:30 and 8:30 a.m.). At the end of the study, a comprehensive blood chemistry test was done for each participant followed by a self-appraisal questionnaire completed by the participant.

Laboratory analysis

To evaluate the effect of the products on the cell-mediated arm of the immune system, the following T-cell subsets were quantified by three-color flow cytometry: $\gamma\delta$ -T cells (CD3⁺CD4⁻CD8⁻), T-helper (Th) cells (CD3⁺CD4⁺CD8⁻), cytotoxic T (Tc) cells (CD3⁺CD4⁻CD8⁺), CD4/CD8 double-positive (DP) T cells (CD3⁺CD4⁺CD8⁺), and Th to Tc cell ratio (Th/Tc). Lymphocytes were counted using a hemocytometer (model MEK6318, Nihon Koden, Tokyo, Japan). Surface phenotypes of the cells were identified by monoclonal antibodies in conjunction with three-color immunofluorescence tests. All monoclonal antibodies used in this study were purchased from Beckman Coulter (Fullerton, CA): PC5 or fluorescein isothiocyanate-anti-CD3 antibody, fluorescein isothiocyanate-anti-CD4 antibody, and phycoerythrin-anti-CD8 antibody. Optilyse® C

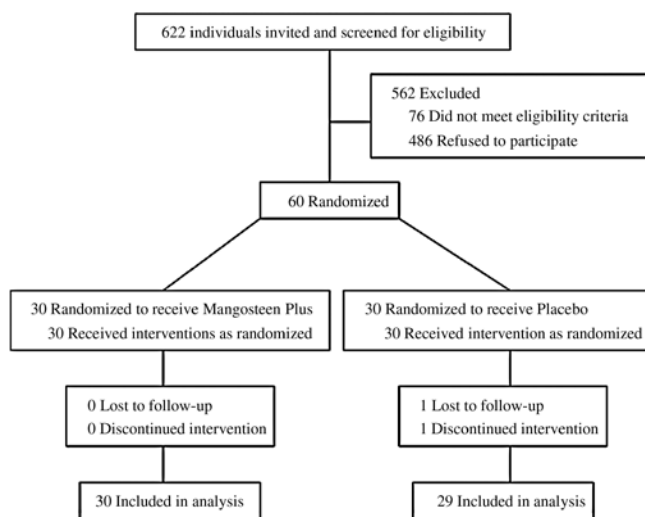


FIG. 1. Flow of participants through the phases of the study.

(Beckman Coulter) was used to lyse the red blood cell. Flow cytometry analysis was performed on a Beckman Coulter EPICS ELITE high-speed cell sorter using the EXPO32 software provided with the instrument. Fluorochrome-isotype-matched controls were prepared to assess auto-fluorescence and nonspecific binding, and single-color samples were used to adjust color compensation.

Serum total immunoglobulins (Igs) (IgG, IgA, and IgM), complement components (C3 and C4), and CRP were detected by using nephelometry (Array 360 Analyzer, Beckman). Serum interleukins (ILs) (IL-1 α , IL-1 β , and IL-2) were measured using commercially available immunoassay kits (Quantikine, catalog numbers DLA50, DLB50, and D2050, respectively; R&D Systems, Minneapolis, MN). Serum concentrations of triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and glucose were determined by the standard protocols.

Statistical analysis

Nominal *P* values are presented. *P* < .05 is considered a statistically significant difference. Normal distribution was assessed by the D'Agostino and Pearson omnibus normality test. All analyses were performed using SPSS software version 10.0 (SPSS Inc, Chicago, IL) after excluding the obviously abnormal values of each index. All continuous variables are represented as mean \pm SD values. Differences between the two groups were compared by the Independent Sample *t* test at baseline and at the end of the study period. The changes of the indices from baseline were calculated and compared between the two groups by the same method. If the variance was not equal, the nonparametric Mann-Whitney test was used for comparisons. Differences between before and after consumption of the test juice were analyzed by the Paired Sample *t* test. The χ^2 test was used to analyze the data obtained from the subjects' self-report questionnaire.

RESULTS

Among the 60 subjects recruited, one was withdrawn during the study because of an employment change. All other participants strictly followed the study protocol and the time schedules during the study period. According to the participants' own reports, investigators' daily observation, and the results of physical examination, no adverse events occurred in both groups.

Baseline profiles

The baseline characteristics of the remaining 59 subjects in both the treatment and the placebo groups are shown in Table 2. At baseline, the two groups did not differ significantly in terms of anthropometric measures, T-cell subsets, ILs, Igs, complement components, and CRP.

Changes in T-cell subsets

Changes in T-cell subsets were observed and shown in Figure 2A. After the intervention, the frequency of periph-

eral blood Tc cells and $\gamma\delta$ -T cells decreased and the Th cell frequency increased in both groups. As a result, the Th/Tc ratio increased. However, the increase was significant only in the experimental group (*P* = .020). Interestingly, at the end of the study, the frequency of DP T cells was increased by 0.28% in the treatment group, whereas it was decreased by 0.18% in the placebo group, resulting in a significant difference between the two groups (*P* = .038, Fig. 2B).

Changes in cytokine production

Figure 3A illustrates that the changes in concentrations of serum IL-1 α , IL-1 β , and IL-2. Compared to baseline values, all three concentrations increased. At the end of the study period, concentrations of IL-1 α and IL-1 β in the study group were significantly higher than those of the control group (IL-1 α , *P* = .033; IL-1 β , *P* = .040). However, only the changes

TABLE 2. BASELINE CHARACTERISTICS OF PARTICIPANTS ASSIGNED TO THE MANGOSTEEN PRODUCT AND PLACEBO LIQUID

| Characteristic | Mangosteen (n = 30) | Placebo (n = 29) | P value ^a |
|--------------------------------------|---------------------|------------------|----------------------|
| Age (years) | 52.37 (5.35) | 52.34 (6.86) | .989 |
| Weight (kg) | 65.95 (9.50) | 68.19 (10.87) | .403 |
| Body mass index (kg/m ²) | 24.19 (2.42) | 25.34 (3.13) | .119 |
| SBP (mm Hg) | 124.00 (20.43) | 128.00 (17.88) | .428 |
| DBP (mm Hg) | 79.73 (13.96) | 79.21 (9.29) | .866 |
| Heart rate (beats/minute) | 66.47 (7.52) | 70.07 (13.49) | .208 |
| Cholesterol (mmol/L) | | | |
| Total | 5.26 (1.05) | 5.13 (0.88) | .608 |
| LDL | 3.36 (0.97) | 3.29 (0.77) | .743 |
| HDL | 1.25 (0.30) | 1.27 (0.29) | .753 |
| Triglycerides (mmol/L) | 1.77 (1.40) | 1.46 (0.68) | .286 |
| Glucose (mmol/L) | 4.83 (0.50) | 4.97 (1.09) | .532 |
| T-cell subsets (%) | | | |
| Total | 70.43 (11.99) | 71.51 (9.75) | .707 |
| $\gamma\delta$ -T cells | 4.92 (4.83) | 3.84 (3.20) | .316 |
| DP T cells | 1.71 (1.52) | 1.76 (2.26) | .917 |
| Th cells | 37.57 (8.87) | 37.22 (9.78) | .884 |
| Tc cells | 26.22 (8.26) | 28.69 (9.50) | .292 |
| Th/Tc ratio | 1.58 (0.65) | 1.54 (0.98) | .831 |
| Cytokines (pg/mL) | | | |
| IL-1 α | 2.92 (2.01) | 4.07 (2.17) | .267 |
| IL-1 β | 2.65 (0.92) | 2.53 (0.74) | .826 |
| IL-2 | 13.55 (6.80) | 14.49 (6.11) | .579 |
| Igs and complement (g/L) | | | |
| IgG | 12.04 (2.65) | 11.53 (2.28) | .433 |
| IgA | 2.26 (1.05) | 2.18 (0.74) | .712 |
| IgM | 1.40 (0.58) | 1.37 (0.63) | .825 |
| C3 | 0.93 (0.20) | 0.91 (0.18) | .733 |
| C4 | 0.21 (0.05) | 0.22 (0.05) | .538 |
| CRP (mg/L) | 1.41 (1.26) | 1.94 (2.03) | .245 |

Data are normally distributed and are mean (SD) values. DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

^a*P* values are calculated by unpaired two-tailed *t* test.

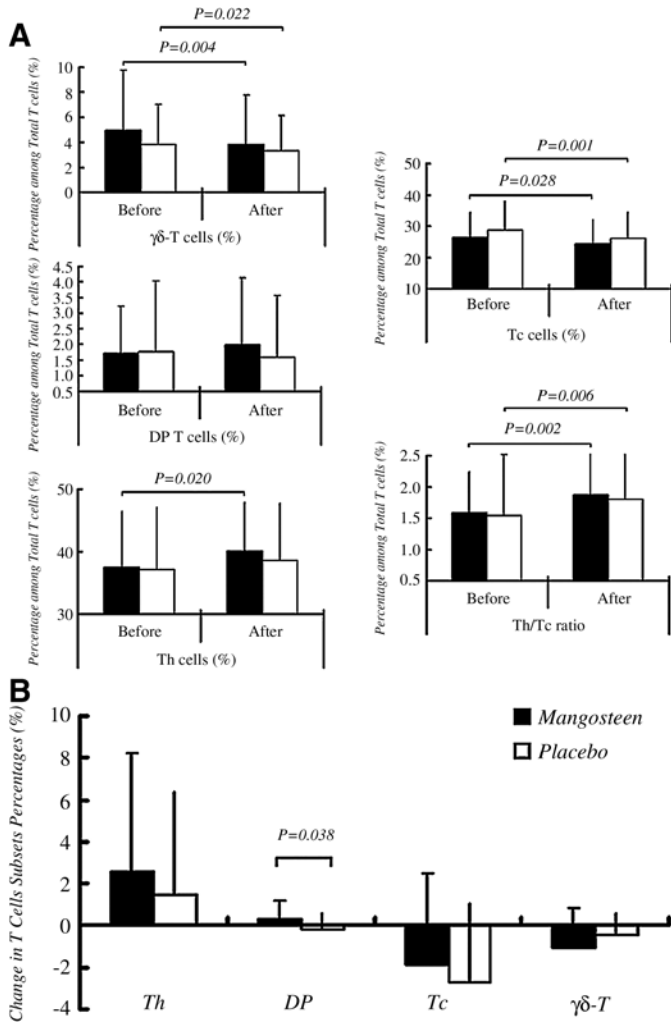


FIG. 2. Changes in T-cell subset percentages before and after the interventions. The mangosteen group consisted of 15 men and 15 women; the placebo group consisted of 14 men and 15 women. (A) Mean (\pm SD) percentage of $\gamma\delta$ -T cells, DP T cells, Th cells, and Tc cells among total T cells and the Th/Tc cell ratio before and after intake of a mangosteen product or placebo. (B) Changes in mean (\pm SD) percentage of T-cell subsets for $\gamma\delta$ -T cells, DP T cells, Th cells, and Tc cells.

in serum IL-1 α concentration were significantly different between the two groups ($P = .006$, Fig. 3B).

Changes in the humoral immune system

Concentrations of serum IgA and complement C3 concentration were compared with their baseline values in both treatment and placebo groups. IgA and C3 concentrations were increased, yet complement C4 concentration was increased only in the study group (Fig. 4). Moreover, both complement components C3 and C4 increased more significantly in the experimental group than in the control group (C3, +0.18 g/L vs. +0.09 g/L, $P = .017$; C4, +0.02 g/L vs. -0.003 g/L, $P = .031$; Fig. 4B).

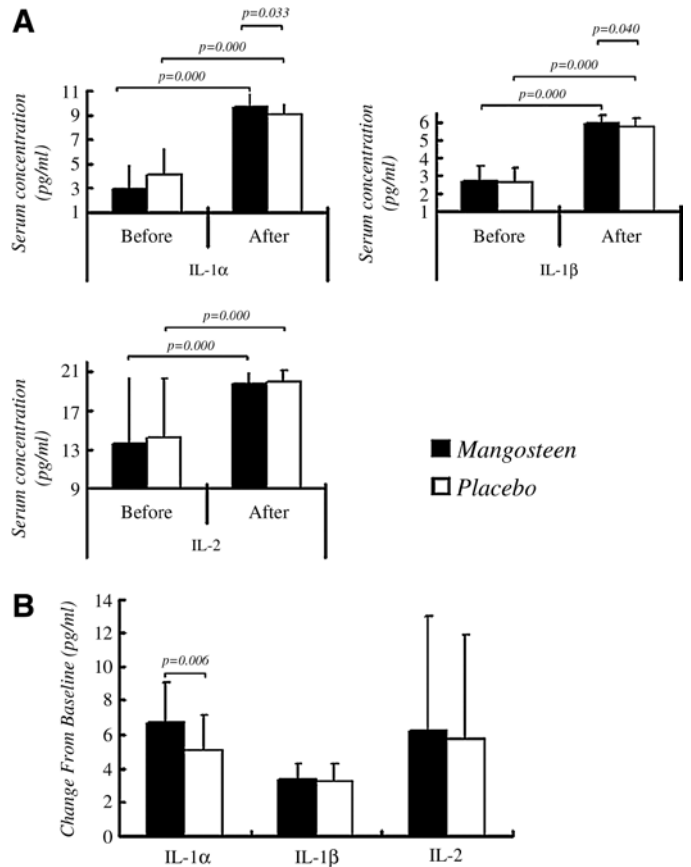


FIG. 3. Changes in serum cytokine concentrations before and after the interventions. The mangosteen group consisted of 15 men and 15 women; the placebo group consisted of 14 men and 15 women. (A) Mean (\pm SD) concentrations of serum IL-1 α , IL-1 β , and IL-2 before and after intake of a mangosteen product or placebo. (B) Changes in mean (\pm SD) concentrations of the serum cytokines IL-1 α , IL-1 β , and IL-2.

Changes in serum CRP concentration

As shown in Figure 5, serum CRP concentration was decreased significantly from 1.32 ± 1.20 mg/L to 0.93 ± 0.79 mg/L ($P = .014$) after consumption of the mangosteen product for 30 days, whereas no significant change was observed in the control group ($P = .429$).

Subjects' self-reported improvement

According to the subjects' self-report, 100% of the participants in the treatment group reported improvement in health status after the intake of mangosteen product versus 75.9% of the participants in the control group ($P = .001$). Furthermore, among those who reported the improvement, only 31.8% in the control group experienced significant improvement versus 76.7% in the treatment group ($P = .001$, Fig. 6). Other specific subject health problems were also analyzed by the same way, among which it was noteworthy that three male volunteers in the treatment group with prostate problems reported feeling much better urinating after drinking the product for a week.

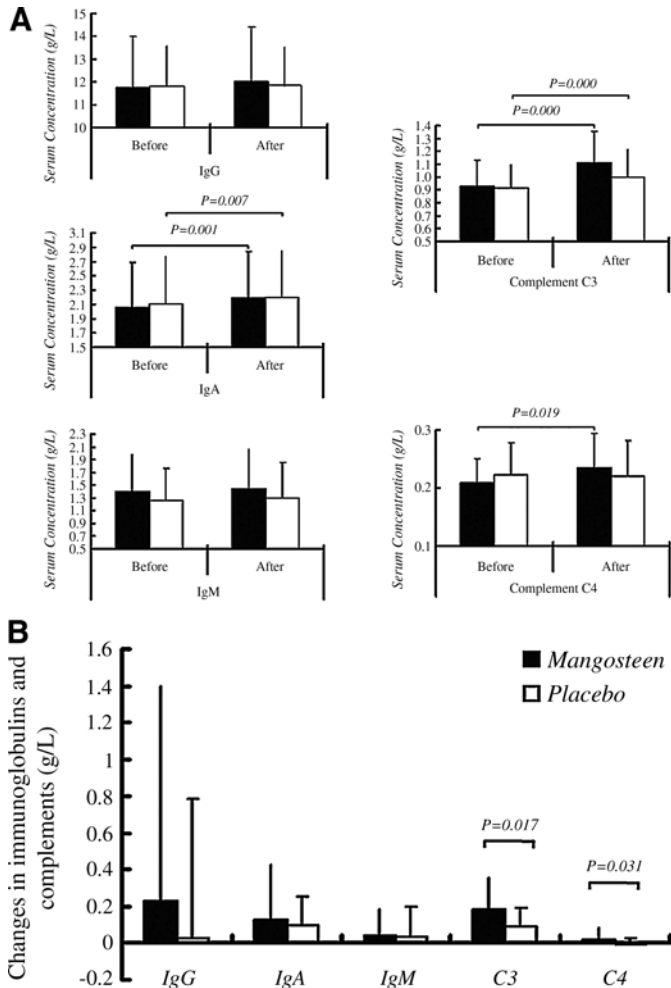


FIG. 4. Changes in humoral immunity before and after the interventions. The mangosteen group consisted of 15 men and 15 women; the placebo group consisted of 14 men and 15 women. (A) Mean (\pm SD) concentrations of serum IgG, IgA, and IgM and complement components C3 and C4 before and after intake of a mangosteen product or placebo. (B) Changes in mean (\pm SD) concentrations of serum IgG, IgA, and IgM and complement components C3 and C4.

DISCUSSION

In this randomized controlled trial, we demonstrated for the first time that intake of a xanthone-rich mangosteen product elevated of the frequency of peripheral Th cells and DP cells and serum IL-1 α , IL-1 β , and complement C3 and C4 concentrations in generally healthy adults without inducing any adverse effects. At the same time, the serum CRP level was suppressed, and subjects' self-appraisals indicated their overall well-being had increased. Our findings suggested that consumption of a xanthone-rich antioxidant supplement product may be able to enhance the immune system within the normal range and exert beneficial effects on overall health status in humans.

As we know, in addition to aging, mild malnutrition and excessive oxidative stress may exert unfavorable effects on immune balance in the long run. For example, inadequate

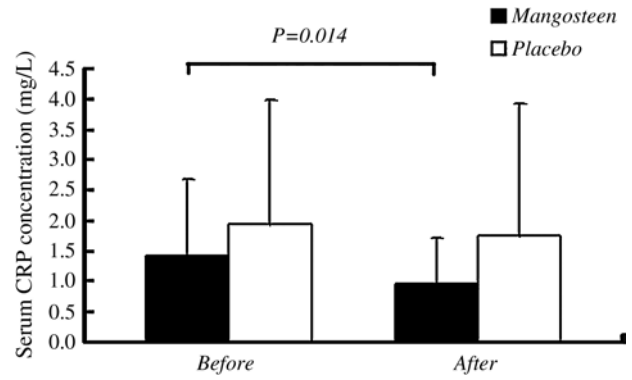


FIG. 5. Changes in mean (\pm SD) serum CRP concentration before and after the interventions. The mangosteen group consisted of 15 men and 15 women; the placebo group consisted of 14 men and 15 women.

intake of protein and various micronutrients such as iron, zinc, vitamin B₁₂, and folate may cause a decrease in monocyte/macrophage activity, Ig deficiencies, and reduction of serum concentrations of cytokines and complement components. Moreover, it has been demonstrated that induction of oxidative stress by glutathione depletion interferes with Th1 immunity, IL-12 production, and costimulatory receptor expression in dendritic cells, leading to decreased interferon- γ production, whereas N-acetylcysteine administration reversed the decline of the delayed-type hypersensitivity response in aged animals.⁹ In line with these studies, dietary supplementation enriched with nutritional doses of vitamins C, E, zinc, selenium, and β -carotene has been reported to restore redox balance and to improve the cell-mediated immunity in animals and in

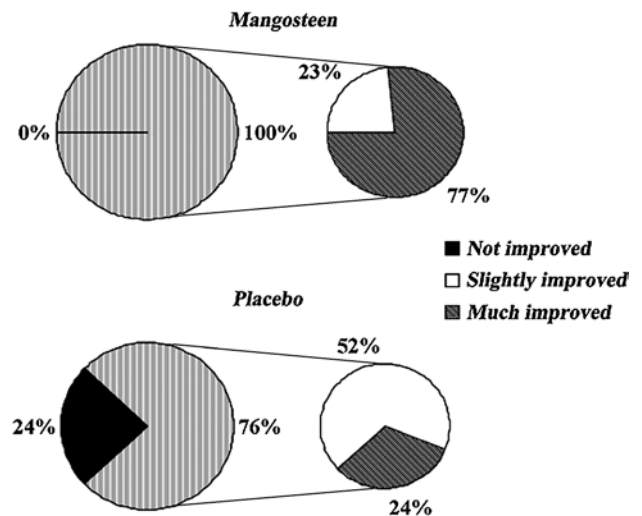


FIG. 6. Subjects' self-reported improvement in overall health status after the interventions. The mangosteen group consisted of 15 men and 15 women; the placebo group consisted of 14 men and 15 women. (Left) Pie charts show percentage of participants who felt their health status improved after the intervention. (Right) Pie charts illustrate that percentage of degree in health improvement of all the patients who reported improvement.

humans.^{7,8,10,11} In this respect, our findings were in accordance with these previous studies.

It was notable that in the present study the peripheral Th cell frequency significantly increased 2.6% (SD 5.7%) after consumption of the mangosteen juice for 30 days (Fig. 2A). Furthermore, the increase in the peripheral DP cell frequency (+0.3%) was significantly higher than that in the placebo group (Fig. 2B). If these results were not caused by a flow cytometry artifact or by physiological fluctuations in T-cell subpopulation, they would be of great value to the enhancement of cellular immunity. On one hand, it has been suggested that low levels of CD4⁺ cells and an increase in CD8⁺ cells are associated with the aging process and are predictive of mortality in the elderly.^{2,3,32} On the other hand, it had been reported that the origin, function, and role of circulating DP cells are mature memory cells. They exhibit typical CD4 and CD8 T-cell functions and mount rapid Th1/Tc1 recall responses to vaccine and viral antigens from self-limited, past, or highly replicative persistent infections, secreting Th1/Tc1 cytokines in response to major histocompatibility class II- and class I-restricted antigens. In addition, the perforin and granzyme A contents of DP cells were comparable to those of CD8 single-positive cells, suggesting that the DP cell population may lead to rapid lysis of virus-infected cells.³³ Thus, overall these results suggested that in the current study consumption of a xanthone-rich product may enhance the acquired immune responses in subjects.

In accordance with the changes in the T-cell subsets, cytokine production also increased in the study group (Tables 3 and 4). In particular, the serum concentrations of IL-1 α and IL-1 β were significantly different between groups after interventions (Fig. 3A). IL-1 plays an important role in immune functions. Its biological effects range from inducing specific cell type responses to targeting entire systems. Although inappropriately dramatic or prolonged production of IL-1 has been implicated as playing a role in the production of a variety of pathological conditions in many diseases, normal or enhancement of IL-1 production is critical to mediation of normal or enhanced host responses to injury and infection as in the current study.³⁴

Figure 4 showed that humoral immunity in the experimental group was also elevated as demonstrated by the augmented concentrations of serum Igs and complements; among these findings, the increases in complement C3 and C4 levels were significantly different between groups (Fig. 4B). Considering that the complement system plays crucial roles in host protection and inflammation as well as in regulation of B lymphocytes of adaptive immunity,³⁵ appropriate increases in C3 and C4 production within the physiological normal range in the present study could be interpreted as beneficial.

However, in the present study, the change in serum CRP concentration was not consistent with that of IL-1 and complement C3 and C4. As shown in Figure 5, the serum CRP level decreased significantly after the mangosteen product supplementation. CRP is an acute-phase reactant whose levels in human plasma dramatically increase up to a

TABLE 3. CHARACTERISTICS OF PARTICIPANTS AFTER 30 DAYS OF EITHER MANGOSTEEN OR PLACEBO LIQUID INTERVENTIONS

| Characteristic | Mangosteen (n = 30) | Placebo (n = 29) | P value ^a |
|------------------------------|---------------------------|-----------------------------|----------------------|
| SBP (mm Hg) | 119.20 (20.60) | 120.10 (17.50) ^b | .863 |
| DBP (mm Hg) | 80.60 (12.80) | 79.90 (11.40) | .825 |
| Heart rate (beats/minute) | 72.00 (9.00) ^b | 73.30 (11.30) ^b | .624 |
| Cholesterol (mmol/L) | | | |
| Total | 5.18 (1.10) | 5.08 (0.79) | .713 |
| LDL | 3.36 (0.97) | 3.21 (0.73) | .500 |
| HDL | 1.33 (0.30) ^b | 1.43 (0.44) ^b | .274 |
| Triglycerides (mmol/L) | 1.57 (0.97) | 1.49 (0.88) | .738 |
| Glucose (mmol/L) | 4.93 (0.43) | 5.11 (1.14) | .418 |
| T-cell subsets (%) | | | |
| Total | 70.33 (9.22) | 69.62 (8.91) | 0.766 |
| $\gamma\delta$ -T cells | 3.83 (3.94) ^b | 3.36 (2.76) ^b | .595 |
| DP T cells | 1.99 (2.15) | 1.58 (1.97) | .444 |
| Th cells | 40.13 (7.74) ^b | 38.67 (9.07) | .508 |
| Tc cells | 24.37 (7.67) ^b | 26.02 (8.49) ^b | .438 |
| Th/Tc ratio | 1.87 (0.81) ^b | 1.80 (1.31) ^b | .802 |
| Cytokines (pg/mL) | | | |
| IL-1 α | 9.62 (1.14) ^b | 9.06 (0.79) ^b | .033 |
| IL-1 β | 5.97 (0.40) ^b | 5.72 (0.49) ^b | .040 |
| IL-2 | 19.76 (1.09) ^b | 19.96 (1.19) ^b | .515 |
| Iggs and complement (g/L) | | | |
| IgG | 12.27 (2.80) | 11.55 (2.19) | .280 |
| IgA | 2.39 (0.99) ^b | 2.27 (0.76) ^b | .606 |
| IgM | 1.44 (0.61) | 1.41 (0.67) | .821 |
| C3 | 1.11 (0.25) ^b | 1.00 (0.21) ^b | .072 |
| C4 | 0.24 (0.06) ^b | 0.22 (0.06) | .280 |
| CRP (mg/L) | 0.93 (0.79) ^b | 1.74 (2.16) | .078 |

Data are normally distributed and are mean (SD) values. DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

^aP values between-group are calculated by unpaired two-tailed *t* test.

^bP < .05 versus baseline as calculated by paired *t* test.

thousandfold if infection or tissue damage occurs.³⁶ Generally, serum CRP level increases with IL-1 α , IL-1 β , and complement C3 and C4 upon infectious attack.^{36,37} One possible reason for the discrepancy may be that in generally healthy subjects, physiological elevation in the level of IL-1 and complements C3 and C4 may not be potent enough to mediate evident increase in CRP synthesis. Another explanation to this apparent discrepancy may be the presence of a wide variety of other antioxidants in the product, such as vitamins C and E, selenium, and green tea catechins, and a number of studies have described inverse relationships between CRP concentration and fruit and antioxidant intakes as well as circulating concentrations of antioxidants.^{38–50} Collectively, although the consumption of the study product elevated the production of pro-inflammatory cytokines, it was able to scavenge the excess reactive oxygen species generated by the inflammatory response, which prevents the concomitant cells or intracellular components from being damaged. Thus, considering that the increase in reactive oxygen species from inflammation may deplete the pools of

MANGOSTEEN SUPPLEMENT AND IMMUNE FUNCTION

TABLE 4. DIFFERENCES IN THE PARAMETERS AFTER 30 DAYS OF EITHER MANGOSTEEN OR PLACEBO LIQUID INTERVENTIONS

| Characteristic | Mangosteen (n = 30) | Placebo (n = 29) | P value ^a |
|------------------------------|------------------------|---------------------|----------------------|
| SBP (mm Hg) | -4.80 (10.30) | -8.40 (17.40) | .334 |
| DBP (mm Hg) | 0.80 (7.60) | 0.50 (9.00) | .866 |
| Heart rate (beats/minute) | 5.60 (9.70) | 3.30 (8.50) | .338 |
| Cholesterol (mmol/L) | | | |
| Total | -0.20 (1.33) | 0.03 (0.59) | .829 |
| LDL | -0.01 (0.58) | -0.08 (0.62) | .625 |
| HDL | 0.08 (0.13) | 0.16 (0.27) | .138 |
| Triglycerides (mmol/L) | -0.09 (0.62) | -0.05 (0.72) | .400 |
| Glucose (mmol/L) | 0.09 (0.53) | 0.14 (0.40) | .718 |
| T cell subsets (%) | | | |
| Total | -0.10 (6.27) | -1.88 (5.77) | .261 |
| $\gamma\delta$ -T cells | -1.09 (1.91) | -0.48 (1.07) | .139 |
| DP T cells | 0.28 (0.94) | -0.18 (0.73) | .038 |
| Th cells | 2.56 (5.69) | 1.45 (4.92) | .429 |
| Tc cells | -1.85 (4.37) | -2.67 (3.69) | .441 |
| Th/Tc ratio | 0.28 (0.44) | 0.26 (0.47) | .835 |
| Cytokines (pg/mL) | | | |
| IL-1 α | 6.75 (2.36) | 5.04 (2.08) | .006 |
| IL-1 β | 3.32 (0.95) | 3.19 (1.08) | .629 |
| IL-2 | 6.21 (6.81) | 5.72 (6.17) | .776 |
| Igs and complement (g/L) | | | |
| IgG | 0.23 (1.17) | 0.02 (0.76) | .424 |
| IgA | 0.13 (0.30) | 0.09 (0.16) | .613 |
| IgM | 0.04 (0.14) | 0.04 (0.16) | .939 |
| C3 | 0.18 (0.17) | 0.09 (0.11) | .017 |
| C4 | 0.02 (0.06) | 0.00 (0.03) | .031 |
| CRP (mg/L) | -0.38 (0.74) | -0.19 (1.25) | .508 |

Data are normally distributed and are mean (SD) values. DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

^aP values between-group are calculated by unpaired two-tailed *t* test.

antioxidants, replenishment of the pool by increased intake of fruits and vegetables or by supplementation may deserve consideration.

Another phenomenon that should be mentioned was that some markers, such as Tc cells, $\gamma\delta$ -T cells, Th/Tc ratio, IgA, and ILs, showed a similar trend of change in both the experimental and the placebo groups as compared with their baseline value. These may mainly be attributable to the changes in immune function over time.

Finally, in accordance with the alterations in the biological markers, the participants' self-appraisals on their overall health status in the study group also significantly improved as compared to the control group (Fig. 6). Although this was a relatively subjective indicator, it was clinically noteworthy and should not be overlooked.

This study has several limitations. First, the sample size was small, and the intervention period may not be long enough to demonstrate the full spectrum of the effects on health status. For instance, although serum Igs and IL-2 concentrations increased more in the treatment group than in the control group, they did not reach a statistically signifi-

cant level. Second, we have not determined which components or their metabolites were responsible for the corresponding effects observed with the mangosteen product. Previously published studies have shown that supplementation with various vitamins or antioxidants, either alone or in combination, can improve various aspects of the immune responses.¹²⁻¹⁷ A combination of the substances in the study juice was likely responsible for the observed effects in the present study, and their effects may be interrelated, intertwined, or even overlapped. In other words, these effects may not be obtained when they are used separately. However, as we have not measured the associated nutritional biomarkers in the subjects, we could not obtain more detailed and specific information on the relationships between the components of the juice and the observed changes in the immunological markers of interest. In the current study, the study population was very homogeneous in terms of nutritional status and other lifestyle factors. They are representative of ordinary, generally healthy adults. Hence, our results may be valid for individuals who are 40-60 years of age, with a poor nutritional status, but generally healthy.

CONCLUSIONS

In conclusion, the data of the present study indicate that consumption of the micronutrient- and antioxidant-rich mangosteen product up-regulated both the innate and acquired immune responses, yet still within the normal range, in apparently healthy adults and resulted in an enhancement of the quality of life. Though sufficient micronutrients and antioxidants may play important roles in maintaining health, it is hard to change human behavior to stay on a complex diet for the long term and requires continuous counseling. Considering that, our finding is intriguing because adoption of small amounts of a product into the habitual diet is a dietary modification that is easy to continue and therefore may be a promising behavioral approach to improve nutritional and health status. Future studies should evaluate the effects of the xanthone-rich product more elaborately in more populations and evaluate long-term outcomes.

AUTHOR DISCLOSURE STATEMENT

M.K., H.-P.J., Y.K., and B.O. are employees of Brunswick Laboratories. The remaining authors have no competing financial interests.

REFERENCES

1. Miller RA: The aging immune system: primer and prospectus. *Science* 1996;273:70-74.
2. Linton PJ, Dorshkind K: Age-related changes in lymphocyte development and function. *Nat Immunol* 2004;5:133-139.
3. Pawelec G, Barnett Y, Forsey R, et al.: T cells and aging, January 2002 update. *Front Biosci* 2002;7:d1056-d1183.
4. Klimas NG, Koneru AO: Chronic fatigue syndrome: inflammation, immune function, and neuroendocrine interactions. *Curr Rheumatol Rep* 2007;9:482-487.

5. Bassi N, Amital D, Amital H, Doria A, Shoenfeld Y: Chronic fatigue syndrome: characteristics and possible causes for its pathogenesis. *Isr Med Assoc J* 2008;10:79–82.
6. Ahluwalia N, Sun J, Krause D, Mastro A, Handte G: Immune function is impaired in iron-deficient, homebound, older women. *Am J Clin Nutr* 2004;79:516–521.
7. Kahmann L, Uciechowski P, Warmuth S, Malavolta M, Mochegiani E, Rink L: Effect of improved zinc status on T helper cell activation and TH1/TH2 ratio in healthy elderly individuals. *Biogerontology* 2006;7:429–435.
8. Alvarado C, Alvarez P, Puerto M, Gausserès N, Jiménez L, De la Fuente M: Dietary supplementation with antioxidants improves functions and decreases oxidative stress of leukocytes from prematurely aging mice. *Nutrition* 2006;22:767–777.
9. Kim HJ, Barajas B, Chan RC, Nel AE: Glutathione depletion inhibits dendritic cell maturation and delayed-type hypersensitivity: implications for systemic disease and immunosenescence. *J Allergy Clin Immunol* 2007;119:1225–1233.
10. Spears JW, Weiss WP: Role of antioxidants and trace elements in health and immunity of transition dairy cows. *Vet J* 2008;176:70–76.
11. Cetinkale O, Senel O, Bulan R: The effect of antioxidant therapy on cell-mediated immunity following burn injury in an animal model. *Burns* 1999;25:113–118.
12. Webb AL, Villamor E: Update: effects of antioxidant and non-antioxidant vitamin supplementation on immune function. *Nutr Rev* 2007;65:181–217.
13. Lanzillotti JS, Tang AM: Micronutrients and HIV disease: a review pre- and post-HAART. *Nutr Clin Care* 2005;8:16–23.
14. Barringer TA, Kirk JK, Santaniello AC, Foley KL, Michielutte R: Effect of a multivitamin and mineral supplement on infection and quality of life. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2003;138:365–371.
15. Bendich A: Micronutrients in women's health and immune function. *Nutrition* 2001;17:858–867.
16. McKay DL, Perrone G, Rasmussen H, et al.: The effects of a multivitamin/mineral supplement on micronutrient status, antioxidant capacity and cytokine production in healthy older adults consuming a fortified diet. *J Am Coll Nutr* 2000;19:613–621.
17. High KP: Micronutrient supplementation and immune function in the elderly. *Clin Infect Dis* 1999;28:717–722.
18. Bardia A, Tleyjeh IM, Cerhan JR, et al.: Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis. *Mayo Clin Proc* 2008;83:23–34.
19. Chong EW, Wong TY, Kreis AJ, Simpson JA, Guymer RH: Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 2007;335:755.
20. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C: Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007;297:842–857.
21. Harman D: Free radical theory of aging: an update: increasing the functional life span. *Ann N Y Acad Sci* 2006;1067:10–21.
22. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD: Antioxidant xanthenes from the pericarp of *Garcinia mangostana* (mangosteen). *J Agric Food Chem* 2006;54:2077–2082.
23. Weecharangsan W, Opanasopit P, Sukma M, Ngawhirunpat T, Sotanaphun U, Siripong P: Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.). *Med Princ Pract* 2006;15:281–287.
24. Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD: Xanthenes with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (mangosteen). *Phytochemistry* 2008;69:754–758.
25. Chen LG, Yang LL, Wang CC: Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food Chem Toxicol* 2008;46:688–693.
26. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W: Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. *Fitoterapia* 2007;78:401–408.
27. Voravuthikunchai SP, Kitpipit L: Activity of medicinal plant extracts against hospital isolates of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2005;11:510–512.
28. Rassameemasmaung S, Sirikulsathean A, Amornchat C, Hirunrat K, Rojanapanthu P, Gritsanapan W: Effects of herbal mouthwash containing the pericarp extract of *Garcinia mangostana* L on halitosis, plaque and papillary bleeding index. *J Int Acad Periodontol* 2007;9:19–25.
29. Nakagawa Y, Iinuma M, Naoe T, Nozawa Y, Akao Y: Characterized mechanism of alpha-mangostin-induced cell death: caspase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells. *Bioorg Med Chem* 2007;15:5620–5628.
30. Devi Sampath P, Vijayaraghavan K: Cardioprotective effect of alpha-mangostin, a xanthenes derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. *J Biochem Mol Toxicol* 2007;21:336–339.
31. Chairungsrilerd N, Furukawa K, Ohta T, Nozoe S, Ohizumi Y: Histaminergic and serotonergic receptor blocking substances from the medicinal plant *Garcinia mangostana*. *Planta Med* 1996;62:471–472.
32. Olsson J, Wikby A, Johansson B, Löfgren S, Nilsson BO, Ferguson FG: Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. *Mech Ageing Dev* 2000;121:187–201.
33. Nascimbeni M, Shin EC, Chiriboga L, Kleiner DE, Rehermann B: Peripheral CD4⁺CD8⁺ T cells are differentiated effector memory cells with antiviral functions. *Blood* 2004;104:478–486.
34. Dinarello CA, Wolff SM: The role of interleukin-1 in disease. *N Engl J Med* 1993;328:106–113.
35. Barrington R, Zhang M, Fischer M, Carroll MC: The role of complement in inflammation and adaptive immunity. *Immunol Rev* 2001;180:5–15.
36. Pepys MB, Hirschfield GM: C-reactive protein: a critical update. *J Clin Invest* 2003;111:1805–1812.
37. Galve-de Rochemonteix B, Wiktorowicz K, Kushner I, Dayer JM: CRP increases production of IL-1 alpha, IL-1 beta, and TNF-alpha and expression of mRNA by human alveolar macrophages. *J Leukoc Biol* 1993;53:439–445.
38. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH: Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. *Am J Clin Nutr* 2006;83:567–574.
39. Schorah CJ: Blood vitamin concentrations during the acute-phase response. *Crit Care Med* 1993;21:1615–1616.

MANGOSTEEN SUPPLEMENT AND IMMUNE FUNCTION

40. Ford ES, Liu S, Mannino DM, Giles WH, Smith SJ: C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. *Eur J Clin Nutr* 2003;57:1157–1163.
41. Gariballa S, Forster S: Effects of acute-phase response on nutritional status and clinical outcome of hospitalized patients. *Nutrition* 2006;22:750–757.
42. Boosalis MG, Snowdon DA, Tully CL, Gross MD: Acute phase response and plasma carotenoid concentrations in older women: findings from the nun study. *Nutrition* 1996;12:475–478.
43. Talwar D, Ha TK, Scott HR, *et al.*: Effect of inflammation on measures of antioxidant status in patients with non-small cell lung cancer. *Am J Clin Nutr* 1997;66:1283–1285.
44. Root MM, Hu J, Stephenson LS, Parker RS, Campbell TC: Determinants of plasma retinol concentrations of middle-aged women in rural China. *Nutrition* 1999;15:101–107.
45. Curran FJ, Sattar N, Talwar D, Baxter JN, Imrie CW: Relationship of carotenoid and vitamins A and E with the acute inflammatory response in acute pancreatitis. *Br J Surg* 2000;87:301–305.
46. Upritchard JE, Sutherland WH, Mann JI: Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* 2000;23:733–738.
47. Kritchevsky SB, Bush AJ, Pahor M, Gross MD: Serum carotenoids and markers of inflammation in nonsmokers. *Am J Epidemiol* 2000;152:1065–1071.
48. Stephensen CB: Vitamin A, infection, and immune function. *Annu Rev Nutr* 2001;21:167–192.
49. Stephensen CB, Gildengorin G: Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the Third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 2000;72:1170–1178.
50. Erlinger TP, Guallar E, Miller ER III, Stolzenberg-Solomon R, Appel LJ: Relationship between systemic markers of inflammation and serum beta-carotene levels. *Arch Intern Med* 2001;161:1903–1908.