



IN THIS EDITION

- FDA and FTC Have Adequate and Substantial Enforcement Authority Over Dietary Supplements
- Chronic Fatigue Syndrome-An Overview and Update
- Phytonutrients and Metabolic Stimulants as Protection Against Neurodegeneration and Excitotoxicity
- The Pharmacology of Saw Palmetto in Treatment of BPH
- Nutraceuticals for Your Pet
- Standardization of Herbal Medicines: The Pandora's Box of Quality Control

AND MORE

A Forum for Wellness and Optimal Health

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American
Nutraceutical
Association**

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To order reprints of articles, or additional copies of *JANA*, write to or call:
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Integrative Medicine and Nutraceuticals – A Physician's Perspective

I am often asked the question: "What exactly IS integrative medicine?" Whether it is a current or potential patient, another physician, or someone who is struggling to keep pace of the swift institutional changes that we are witnessing, many are perplexed by this new term.

The more common terms "alternative medicine" or complementary therapies" have become more comfortable to us as the lines of agreement and disagreement are drawn with more definition. But as expectations for our level of function at any given age increase, so will the bandwidth change of our definition of health and its maintenance. When we hear of someone's life being foreshortened at the tender age of 68, for example, we feel a different sense of tragedy and missed fulfillment than we would have 20 years ago.

In 1978--the year I completed my residency training at the University of Minnesota Hospitals--a terminal illness in the seventh decade of life was treated with a combination of concern and equanimity as most felt that a "full life had been lived". Contrast that with the anticipated deliverables of the modern day octogenarian. Independent living filled with the pursuits of art, recreational and competitive athletics, and even meaningful careers is no longer exceptional. How does a healthcare system that is more and more stingy over what it delivers accommodate the needs of this burgeoning demographic group? Some of the answers are being supplied by providers of health strategies that involve the tools of functional medicine: nutraceuticals. How these strategies are interwoven into the case management of modern healthcare necessitates the kind of thinking that is becoming known as "integrative medicine" or more accurately, "integrative health".

How would an integrative health practitioner approach a present day case of coronary heart disease, for example, that might distinguish it from any other approach? First, there is the belief that authority is a shared responsibility. This single element may be the underlying force behind the alternative-complementary-holistic-integrative evolution. Before the tactics of mind-body medicine, acupuncture, nutraceuticals, or homeopathy are involved, there is the initial principle of shared authority. This belief has likely driven the rise of integrative health all along. In an age of deregulated information, authority can no longer be held by the provider alone.

With such shared decision-making comes the second guiding principle: one size does not fit all. Each patient arrives at certain health "waypoints" with a unique combination of gene expression, environmental influences, and beliefs. All cases of atherosclerosis are not created equally. One person's folic acid or pyridoxine requirements might be ten or twenty times that of a second patient's--and they might

both have nearly identical three-vessel disease on an angiogram. Cases that appear to be identical require vastly different approaches when seen through the lens of the integrative practitioner. We see the hazards of this more and more as we "pathway" and genericize our patient populations to the point where very few treatments are customized ideally to the patient's needs and individual gene expression.

We are finally evolving out of the strict "one disease, one drug" approach to health and illness. Are we really in an optimal state of health until we catch a codifiable disease? Or are we continually growing and operating along a functional continuum the nature of which is determined by a complex set of variables including specific nutrient supplements. Nutraceutical augmentation provides one of the essential toolboxes for integrative practitioners as we move forward and reinvent the concept of "managing one's health".

Finally, integrative health and medicine requires a change in attitudes and beliefs. We are not necessarily the unknowing victims of a fated script that sends some of us to the hospital many times before the age of 50 and leaves others conspicuously free of disease despite some rather worldly habits. Rather we can learn to influence and direct our genetic software for illness becomes a better bet for all of us.

Integrative health and medicine may become much more than all of this as information is networked and authority is shared at the patient level. The American Nutraceutical Association is determined to provide the necessary stewardship of that information so good decisions can be made by all as we "integrate" our healthcare.

I am pleased to announce that beginning in 2000, *JANA* will be published quarterly. The first edition will focus on the dangers and public health risks of obesity and the evaluation of herbal products used for weight loss. The obesity issue will include the results of a 6-month clinical study conducted by Gary L. Huber, MD. The clinical study compared Ephedra-caffeine herbal products with pharmaceutical products for safety and efficacy. Other *JANA* editions in 2000 will focus on nutraceuticals used for cardiovascular health, osteoarthritis, migraine, diabetes, and other medical conditions. We are also adding a new section on "nutraceuticals for animals" that will be coordinated by Todd Henderson, DVM.

Finally, don't forget to mark your calendars...the second ANA sponsored "Nutraceuticals and Medicine Conference" is scheduled for March 31-April 1, 2000 in San Diego (see pg 13).

On behalf of coeditor, Allen Kratz, PharmD and myself, I extend to you our best wishes for the New Year!

Christopher M. Foley, MD
Coeditor



Journal of the American Nutraceutical Association

Continuing Education Program

Vol. 2, No.3

To Our Healthcare Professional Readers:

Four articles in this edition of *JANA* have been selected for a total of two (2) hours of continuing education credits for physicians, pharmacists and nurses. This educational program has been developed as part of the ANA mission to provide science-based information on nutraceuticals and dietary supplements for healthcare professionals. One goal of this program is to continually assess the educational needs of *JANA* readers. Your evaluation of this educational program will help us achieve this goal.

Christopher M. Foley, MD

Allen M. Kratz, PharmD

Coeditors, *Journal of the American Nutraceutical Association*

CME, CPE, and CNE Program

Physicians with current and valid licenses in the US and Canada are eligible for two (2) hours of Category 1 CME, based on the material in the selected articles. Licensed pharmacists will receive two (2) hours (0.2CEUs) of credit, and licensed nurses will receive two (2) contact hours CNE. To proceed, simply read the articles selected by the continuing education advisory board for CME, CPE, and CNE; complete the test and evaluation form, and return it by mail, fax, or Email within twelve months of receiving the journal.

Statement of Educational Purpose

The goal of this program is to offer healthcare professionals (physicians, pharmacists and nurses) the opportunity to develop knowledge, understanding and competency in the use of nutraceuticals. This continuing education program provides access to the latest peer-reviewed studies on nutraceuticals, information that can be used to optimize their patients' and their own health and quality of life.

Objectives for *JANA* Volume 2, Number 3

After reading the selected articles in this edition of *JANA*, and thoughtfully considering the information provided and discussing it with colleagues, a physician, nurse and pharmacist will be able to:

1. Identify the demographics and diagnostic criteria for Chronic Fatigue Syndrome, and discuss treatment options and protocols for CFS.
2. Describe the molecular mechanism known as excitotoxicity and identify the roles that various nutraceuticals play in neuroprotection.
3. Identify the pharmacological mechanism of saw palmetto in the treatment of BPH and compare the treatment options of saw palmetto with those of prazosin, terazosin, and finasteride.
4. Identify the effects of *Aphanizomenon flos aquae* (AFA) on the circulation and function of immune cells as demonstrated in the clinical study presented, and describe the design and methodology of the research study utilized to establish the roles of AFA in immune modulation.

CME, CPE, CNE Articles

1. Chronic Fatigue Syndrome—An Overview and Update (page 23)
2. Phytonutrients and Metabolic Stimulants as Protection Against Neurodegeneration and Excitotoxicity (page 30)
3. The Pharmacology of Saw Palmetto in Treatment of BPH (page 40)
4. Consumption of *Aphanizomenon flos aquae* Has Rapid Effects on the Circulation and Function of Immune Cells in Humans (page 50)

Disclosure

Mary O'Brien, MD - No financial interests; Russell Blaylock, MD - No financial interests;
Marilyn Barrett, PhD - No financial interests; Gitte S. Jensen, PhD - No financial interests.

Receiving Credit

After reading the material, complete the test and evaluation form and return it to the address listed on the form. Passing grade of 70% is required in order to obtain continuing education credits. A certificate awarding two (2) hours of Category 1 CME for physicians, two (2) hours (.02 CEUs) for pharmacists, and two (2) contact hours for nursing will be faxed or mailed to you. If your certificate becomes lost, physicians and nurses should contact the Foundation for Care Management office in Kirkland, Washington (425-820-9655). Pharmacists should contact the College of Pharmacy, Medical University of South Carolina in Charleston, South Carolina (843-792-3113). Your record will be kept on file by these institutions for seven years.

Advisory Board for Continuing Education Program

Co-Chairs: Stephen Yarnall, MD, FACC, FACP, Stevens Health Center and medical director, Foundation for Care Management; Carole A. Mutzebaugh, NP, EdD, associate director, Foundation for Care Management, former Dean, School of Nursing, University of Southern Colorado; Ronald Nickel, PhD, Associate Professor of Pharmacy Practice and director of continuing pharmacy education, College of Pharmacy - Medical University of South Carolina.

Accreditation:

The Foundation for Care Management (FCM) is an organization accredited by the Washington State Medical Association Medical Education Committee to provide continuing medical education. FCM certifies that this course meets the criteria for two (2) hours of Category 1 CME to satisfy relicensure requirements and the Physicians Recognition Award of AMA (PRA). FCM is approved as a provider of continuing education in nursing by the Colorado Nurses Association, which is accredited as an approver of CNE by the American Nurses Credentialing Center's Commission on Accreditation. FCM has approved this program for two (2) contact hours of CNE.



The Medical University of South Carolina College of Pharmacy is approved by the American Council on Pharmaceutical Education as a provider of continuing pharmaceutical education. This program for pharmacists is approved by the MUSC College of Pharmacy for two (2) hours (0.2 CEUs) of credit. Program # 061-999-99-60-H01.



Continuing Medical, Pharmacy and Nursing Education Test

To earn two (2) hours of Category 1 CME, two (2) hours (0.2 CEUs) of CPE, and two (2) contact hours (CNE), complete the following test and program evaluation form. Fax or mail it to the address below, or complete the form on the Web, (via www.Americanutra.com or www.medicare.org). You need to complete all parts of this form to receive credit. Allow three to four weeks for your certificate to arrive by fax or mail.

Part 1. Test: After reading the articles selected by the editorial board for this edition, complete the following test by circling the appropriate answer.

Chronic Fatigue Syndrome—An Overview and Update (page 23)

Author: Mary E. O'Brien, MD, Associate Professor of Medicine, University of North Carolina

Objective: Identify the demographics and diagnostic criteria for Chronic Fatigue Syndrome. Discuss treatment options and protocols for CFS.

- (1) In 1994, the consensus criteria for the diagnosis of CFS was updated. The current diagnosis of CFS requires clinically unexplained fatigue that has persisted for:
 - A. 2 months
 - B. 6 months
 - C. 8 months
 - D. 12 months

- (2) The fatigue must not be related to exertion nor relieved by rest and results in significant reduction in social, personal, educational, and professional levels.
() True () False

- (3) How many of the following symptoms on a concurrent and persistent basis are required for a CFS diagnosis:
 - Impaired concentration or short term memory loss
 - Sore throat
 - Tender cervical or auxiliary lymph nodes
 - Arthralgia without arthritis
 - Headaches new in character, severity, or pattern
 - Unrefreshing sleep
 - Post-exertional fatigue that lasts for more than 24 hours
 - A. 2
 - B. 4
 - C. 6
 - D. All

- (4) Regardless of the etiology, CFA affects more:
() Men () Women



- (5) In one study, CFS patients with low to low-normal carnitine levels improved in 12 of 18 parameters of mental and physical fatigue after 4 to 8 weeks of L-carnitine supplementation. The dose in this trial was:
- A. 500 mg three times a day
 - B. 250 mg twice a day
 - C. 1 gram three times a day

Phytonutrients and Metabolic Stimulants as Protection Against Neurodegeneration and Excitotoxicity (page 30)

Author: Russell Blaylock, MD, Clinical Assistant Professor, University of Mississippi Medical Center

Objective: Describe the molecular mechanism known as excitotoxicity and identify the roles that various nutraceuticals play in neuroprotection.

- (1) With severe injuries to the brain, the amount of glutamate in the extracellular space may reach levels that are:
- A. 20x greater than normal
 - B. 100x greater than normal
 - C. 10x greater than normal
- (2) Recent studies indicate that patients with Parkinson's Disease have a defect in:
- A. iron metabolism
 - B. calcium metabolism
 - C. glutamate metabolism
- (3) In recent studies, it has been shown that exposure to the following early in life can lead to the development of abnormal pathways in the brain, especially in the visual system and hypothalamus:
- A. iron
 - B. MSG
 - C. lead
 - D. free radicals
- (4) Cellular protection can be increased by enhancing glutathione levels. Several methods have been found to accomplish this including the following:
- A. increasing ascorbate levels
 - B. alpha lipoic acid supplementation
 - C. increased intake of precursors such as cystine and n-acetyl-l cysteine
 - D. all of the above

Upon completion of this test please cut along the dotted line and return the test along with the program evaluation form to the Foundation for Care Management.
See page 12 for mailing address and fax number.



The Pharmacology of Saw Palmetto in Treatment of BPH (page 40)

Author: Marilyn Barrett, PhD, Pharmacognosy Consulting Services

Objective: Identify the pharmacological mechanism of saw palmetto in the treatment of BPH and compare the treatment options of saw palmetto with those of prazosin, terazosin, and finasteride.

- (1) Clinical studies have demonstrated that saw palmetto have demonstrated significant improvement in BPH symptoms as scored by the International Prostate System Score (IPSS) when administered at:
 - A. 320 mg of extract daily for 1 to 2 months
 - B. 160 mg of extract daily for two weeks
 - C. 320 mg of extract daily for two weeks
- (2) Clinical trials have found that BPH treatment with saw palmetto resulted in a significant increase in serum levels of prostate specific antigen (PSA).
() True () False
- (3) The suggested pharmacological actions of saw palmetto preparations include:
 - A. anti-androgenic activity (inhibition of the conversion of testosterone to DHT by 5-alpha reductase and inhibition of binding of DHT to androgen receptors)
 - B. anti-inflammatory activity
 - C. anti-proliferative activity
 - D. relaxation of smooth muscle
 - E. all of the above
- (4) In a 6 month clinical trial comparing the efficacy of saw palmetto extract to that of finasteride, which of the following results were found
 - A. both treatments were determined to decrease the symptoms of BPH in about two-thirds of the patients
 - B. finasteride treatment was more effective in decreasing prostatic volume
 - C. none of the above
 - D. both of the above

Consumption of Aphanizomenon flos aquae Has Rapid Effects on the Circulation and Function of Immune Cells in Humans (page 50)

Author: Gitte S. Jensen, PhD, et al.

Objective: Identify the effects of AFA on the circulation and function of immune cells as demonstrated in the clinical study presented, and describe the design and methodology of the research study utilized to establish the roles of AFA in immune modulation.

- (1) In a study to examine the benefits of the blue green algae *Aphanizomenon flos-aquae* (AFA) on the immune system, subjects were given orally:
 - A. 2.5 grams in a single dose
 - B. 2.0 grams twice daily
 - C. 1.5 grams in a single dose
- (2) Volunteers in the study were asked not to consume vitamin preparations or other nutraceuticals:
 - A. 12 hours before the study
 - B. 18 hours before the study
 - C. 24 hours before the study
- (3) This study has shown that consumption of AFA has led to increased trafficking of absolute numbers of lymphocytes and monocytes whereas polymorph nucleated cells, were not affected indicating selected mobilization of immune responses.
() True () False
- (4) Increased trafficking of immune cells should translate into a better immune surveillance, i.e., a more efficient patrolling microbial invaders, as well as virus-infected or transformed cells.
() True () False

Part II. Program Evaluation Form. For each of the relevant articles in this issue, please respond to the following questions by putting a circle around the number you regard as most appropriate for each question, with 5 showing the strongest agreement:

Article:	<u>O'Brien</u>	<u>Blaylock</u>	<u>Barrett</u>	<u>Jensen, et al.</u>
Educational Value:				
I learned something new and valuable	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
I verified some important information	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
I plan to discuss this data with colleagues	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
I plan to seek more insights in this field	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
My attitude about this topic changed	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
This information may impact my practice	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
General Feedback:				
I understood the material	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
I was able to interpret the figures	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
The presentation was helpful	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Overall the journal is helpful	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

Additional comments:

Part III. Possible change. What change(s), if any, do you plan to make in your practice as a result of reading these journal articles? (use additional sheet if more space required)

Part IV. Statement. I attest to completing the CME, CPE or CNE activity.

Please indicate your profession: physician nurse pharmacist

Signed: _____ Date: _____ License # _____

Part V. Identifying information. Please PRINT legibly or type the following:

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Part VI. Payment information. The CME, CPE, CNE fee for this program is US \$15.00.

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ANA Announces Nutraceuticals and Medicine Conference for March 2000

The American Nutraceutical Association (ANA) will sponsor the second annual Nutraceutical and Medicine Conference March 31st and April 1st, 2000, at the Wyndham Emerald Plaza Hotel in San Diego, California

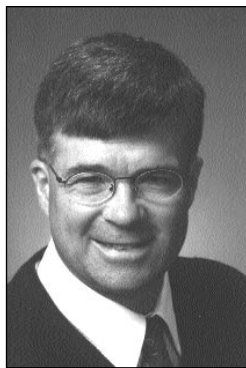
The 2000 conference is planned in cooperation with the Foundation for Care Management based in Kirkland, Washington, and the College of Pharmacy and College of Dental Medicine at the Medical University of South Carolina. The conference will provide health care professionals with the latest scientific data on nutraceuticals and the integration of nutraceuticals into their practices. The conference will award 8 category 1 CME credits to physicians, and 8 contact hours of CE to nurses and pharmacists. The conference agenda has also been submitted to the California Chiropractic Association for approval for California chiropractors.

Christopher M. Foley, MD, co-editor of the *Journal of the American Nutraceutical Association (JANA)*, and Director of Integrative Health, HealthEast Care System, St. Paul, Minnesota, is serving as conference chair. According to Dr. Foley, "Our primary goal is to offer healthcare professionals an opportunity to develop competency in using nutraceuticals in their practices. Interaction among participants and speakers is designed to enrich their knowledge and understanding of how nutraceuticals can be used to optimize their patients' and their own health and quality of life."

"The 1999 conference was a great success," noted Allen Montgomery, RPh, CEO, of the American Nutraceutical Association. "Over 300 persons attended last year's conference, and we expect this year's program to be a sell-out. The speakers include both practitioners and academicians who will provide up-to-date scientific information for health care professionals on the benefits of nutraceuticals."

TOPICS AND SPEAKERS CONFIRMED FOR THE CONFERENCE INCLUDE:

- **"New Developments in Phytoprevention and Treatment of Cancer"** – Russell Blaylock, MD, Neurosurgeon and Clinical Assistant Professor, Medical University of Mississippi



Christopher M. Foley, MD
Conference Chair

- **"Pathogenesis and Prevention of Disease and Aging"** – Richard Dubois, MD, Chief of Medicine – Baptist Hospital, Atlanta, Georgia
- **"Nutraceutical Influences on Gene Expression – How They May Apply in the New Medical Practice"** – Christopher M. Foley, MD – Director of Integrative Health, HealthEast Care System, St. Paul, Minnesota, and co-editor, *JANA*
- **"The Use of Antioxidants in the Prevention and Treatment of Coronary Heart Disease"** – Stephen Rosenblatt, MD, PhD, Diplomate, American Board of Family Practice, former Director of Complementary Medicine, Cedars – Sinai Medical Center and lecturer for Blue Cross of California in complementary medicine
- **"Drug Interactions and Toxicity with Herbs and Nutraceuticals"** – Rick Kingston, PharmD, Assistant Professor, Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, and Vice President and Senior Toxicologist, Prozar International Poison Control Center
- **"In Quest of Wellness and Optimal Longevity – The Emerging Pre-eminence of Nutraceuticals and Dietary Supplements for the New Millennium"**
Gary L. Huber, MD, Director, Texas Nutrition Institute, ETMC Rehabilitation Center, East Texas Medical Center Healthcare System, Tyler, Texas, and Vice President for Scientific Affairs, American Nutraceutical Association
- **"Nutritional and Nutraceutical Support for Patients with Chronic Fatigue Syndrome and Fibromyalgia"**
Mary O'Brien, MD, Associate Professor of Medicine, University of North Carolina, and Director of Geriatric Services, Coastal AHEC

Through this ANA - sponsored conference, participants will have the opportunity to better understand the scientific basis for the use of dietary supplements and nutraceuticals in their healthcare practices and to evaluate the current research in selected categories of nutraceuticals and their effect on health conditions. In addition, participants will learn more about the emerging importance of nutraceuticals in the new millennium and identify potential drug interactions with herbs and nutraceuticals.

- Early Conference registration fee is \$140 per person if received prior to February 1, 2000.
- Conference registration fee after February 1st is \$165 per person.
- To register by phone with a credit card, call the ANA customer service department at 800-566-3622 (outside the USA call 205-833-1750) Monday-Friday, 8:00 AM to 5:00 PM central standard time.
- To register by mail, complete a conference registration form, and mail it along with a check made payable to ANA, 22 Inverness Center Parkway, Suite 150, Birmingham, AL 35242.

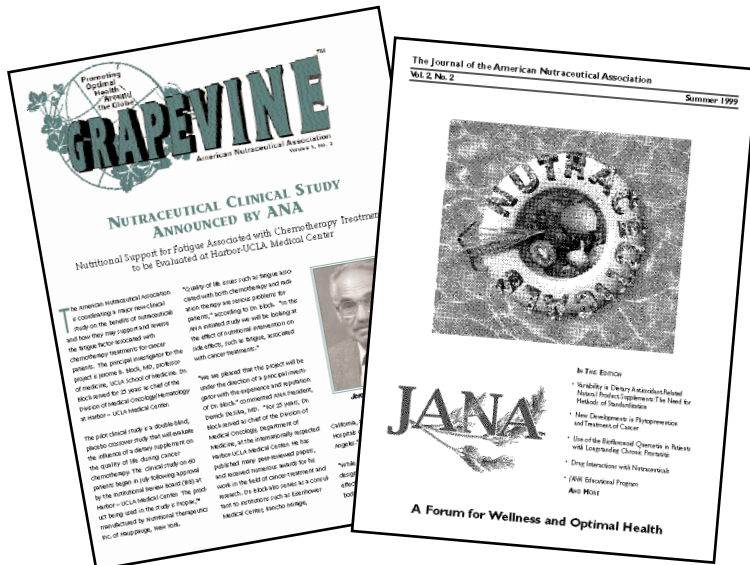
For more information on the 2000 Nutraceuticals and Medicine Conference, or to request a conference brochure and registration form, call the ANA executive office at (205) 980-5710.

Clinical Study on Dietary Supplement for Migraine Prevention Announced by Kaiser-Permanente

An important double-blind, placebo-controlled trial of a combined natural ingredient supplement for migraine will be conducted at Kaiser-Permanente beginning in early 2000. The dietary supplement being evaluated is Migra-Lieve™ which contains magnesium, riboflavin (vitamin B2), and feverfew, all of which have shown in scientific studies to benefit in the prevention of migraines. Patent pending Migra-Lieve™ is the only dietary supplement which combines all 3 compounds. It is currently being used by numerous physicians and clinics including Alexander Mauskop, MD, associate professor of clinical neurology at the State University of New York/Downstate Medical Center in Brooklyn, New York, and director of the New York Headache Center in New York City.

The clinical study on the dietary supplement Migra-Lieve™ is designed to monitor patient's migraine frequency and severity over a 3-month period to determine if the combined supplement is effective. The authors anticipate the study will take 6 - 12 months to complete. The prime investigators from Kaiser-Permanente will be Morris Maizels, MD, Department of Family Practice, Woodland Hills, California, and Jeffrey Cohen MD, Department of Neurology, Denver, Colorado. Both of these physicians have previously published research in the field of migraine.

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Obesity Epidemic Increases Dramatically in the United States: CDC Director Calls for National Prevention Effort

A growing obesity epidemic is threatening the health of millions of Americans in the United States, according to CDC (Centers for Disease Control and Prevention) research published in the October 27, 1999, issue of *The Journal of the American Medical Association (JAMA)*. According to the findings, the obesity epidemic spread rapidly during the 1990s across all states, regions, and demographic groups in the United States. Obesity (defined as being over 30 percent above ideal body weight) in the population increased from 12 percent in 1991 to 17.9 percent in 1998. The highest increase occurred among the youngest ages (18- to 29-year-olds), people with some college education, and people of Hispanic ethnicity. By region, the largest increases were seen in the South with a 67% increase in the number of obese people. Georgia had the largest increase--101%. The findings also show that a major contributor to obesity -- physical inactivity-- has not changed substantially between 1991 and 1998.

"Overweight and physical inactivity account for more than 300,000 premature deaths each year in the U.S., second only to tobacco-related deaths. Obesity is an epidemic and should be taken as seriously as any infectious disease epidemic," says Jeffrey P. Koplan, director of the CDC, and one of the authors of the *JAMA* article. "Obesity and overweight are linked to the nation's number one killer--heart disease-- as well as diabetes and other chronic conditions."

A national effort is needed to control the epidemic, according to Koplan. "While obese individuals need to reduce their caloric intake and increase their physical activity, many others must play a role to help these individuals and to prevent a further increase in obesity," Koplan says. "Health care providers must counsel their obese patients; workplaces must offer healthy food choices in their cafeterias and provide opportunities for employees to be physically active on site; schools must offer more physical education that encourages lifelong physical activity; urban policymakers must provide more sidewalks, bike paths, and other alternatives to cars; and parents need to reduce their children's TV and computer time and encourage outdoor play. In general, restoring physical activity to our daily routines is critical."

According to surveys conducted in 1977-78 and 1994-96, reported daily caloric intakes increased from 2239 Kcal to 2455 Kcal (calories) in men, and from 1534 Kcal to 1646 Kcal in women. Eating more frequently is encouraged by innumerable environmental changes: more food and foods

with higher caloric content, the growth of the fast food industry, the increased numbers and marketing of snack foods, increased time for socializing, and a custom of socializing with food and drink.

At the same time, there are fewer opportunities in daily life to burn calories: children watch more television daily; many schools have done away with or cut back on physical education; many neighborhoods lack sidewalks for safe walking; the workplace has become increasingly automated; household chores are assisted by labor-saving machinery; and walking and cycling have been replaced by automobile travel for all but the shortest distances. According to Koplan, the American lifestyle of convenience and inactivity has had a devastating toll on every segment of society, particularly on children. Research shows that 60% of overweight 5- to 10-year-old children already have at least one risk factor for heart disease, including hyperlipidemia and elevated blood pressure or insulin levels.

According to CDC research published in the October 13, 1999, issue of *JAMA*, more than two-thirds of American adults are trying to lose weight or keep from gaining weight but many do not follow guidelines recommending a combination of fewer calories and more physical activity. The 1996 Surgeon General's Report on Physical Activity and Health shows that more than 60 percent of adults are not participating in the recommended 30 minutes a day of moderate physical activity most days of the week. The Report stresses that physical activity need not be strenuous to achieve health benefits.

The American Nutraceutical Association will publish a special edition of the *Journal of the American Nutraceutical Association (JANA)* during the first quarter of 2000 that will focus on the dangers of obesity and the use of herbal products in a weight loss program. The result of a 6-month study conducted by ANA Vice President for Scientific Affairs, Gary L. Huber, MD, will be published in this obesity-weight loss issue of *JANA*. In his study, Dr. Huber compared 3 herbal weight loss products with pharmaceutical products for safety and efficacy. Two of the herbal products contained a combination of Ephedra and caffeine. The preliminary results of his study were presented at the International Symposium on Ephedra in Washington, D.C., December 9-10, 1999.

For more information about nutrition and physical activity, call toll-free 1-888-CDC-4NRG or visit the CDC website at www.cdc.gov/nccdphp/dnpa.

FDA Finalizes Rules for Claims On Dietary Supplements

The FDA has published its final rule that defines the types of statements that can be made concerning the effect of a dietary supplement on the structure or function of the body pursuant to the Dietary Supplement Health and Education Act of 1994 (DSHEA).

Under DSHEA, dietary supplements may bear "structure/function" claims -- claims that the products affect the structure or function of the body -- without prior FDA review. They may not, without prior FDA review, bear a claim that they can prevent, treat, cure, mitigate or diagnose disease (a disease claim).

This final rule describes how FDA will distinguish disease claims from structure/function claims. While this rule should not affect the availability of dietary supplement products or consumer access to them, it may affect whether certain claims can be made under DSHEA and therefore may result in some labeling changes for these products.

The final rule precludes express disease claims ("prevent osteoporosis") and implied disease claims ("prevents bone fragility in post-menopausal women") without prior FDA review. The final rule clarifies that such express and implied disease claims can be made through the name of a product ("Carpaltum," "CircuCure"), through a statement about the formulation of a product (contains aspirin), or through the use of pictures, vignettes, or symbols (electrocardiogram tracings). The rule permits claims that do not relate to disease. These include health maintenance claims ("maintains a healthy circulatory system"), other non-disease claims ("for muscle enhancement," "helps you relax,"), and claims for common, minor symptoms associated with life stages ("for common symptoms of PMS," "for hot flashes").

In response to comments from industry and consumers, FDA made several significant changes in the final rule. These changes, which have the effect of expanding the number of acceptable structure/function claims, include revising the definition of "disease" in response to comments that it was too broad and permitting structure/function claims about certain common conditions

associated with aging, pregnancy, menopause, and adolescence. Serious conditions associated with aging, pregnancy, menopause, and adolescence, such as toxemia of pregnancy, and osteoporosis, will continue to be treated as diseases.

Under DSHEA and existing regulations, dietary supplement manufacturers are already required to have, in their files, substantiation of any structure/function claims they make. They must also include a disclaimer on their labels that the dietary supplements are not drugs and receive no FDA pre-market approval. Finally, they must notify FDA of the claims they are making within 30 days of marketing a given dietary supplement.

FDA believes that this rule, which clarifies appropriate structure/function claims, will ultimately provide consumers with better information on dietary supplement labeling that will help them select appropriate products. The issuance of this rule is an important part of FDA's overall dietary supplement strategy which is aimed at providing consumers with a high level of confidence in the safety, composition, and labeling of dietary supplements.

The rule, published in the Jan. 6, 2000 Federal Register, will become effective 30 days after the date of publication. Any product that is marketed for the first time after the date of publication and any new claims made for an existing product for the first time after publication will be expected to comply with the rule beginning 30 days after publication. Small businesses that marketed a product as of the publication date will have an additional 17 months to bring existing claims into compliance and all other products that were on the market as of the publication date will have an additional 11 months to bring existing claims into compliance.

For more information visit the Center for Food Safety and Applied Nutrition - Food and Drug Administration's Website at (www.cfsan.fda.gov). On this site, go to the "What's New" page.

FDA and FTC Have Adequate and Substantial Enforcement Authority Over Dietary Supplements

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Five years have elapsed since the passage of the 1994 Dietary Supplement and Health Education Act (DSHEA).¹ Over this time, dietary supplements have essentially been a legislated, not regulated, industry. The Federal Drug Administration (FDA) has shown increasing regulatory attention to dietary supplements, but it was not until 1998-1999 that the agency initiated activity to develop an “overall strategic plan” for this class of consumer products.² That FDA has begun efforts for managing the quality and claims framework for dietary supplements is ultimately good for the industry.

The Over-The-Counter (OTC) medicines industry experienced, with some trepidation, the “imposition” of the OTC Review in 1972³ and with it regulatory standards for safety, effectiveness, and labeling consistent with the expectations of intended therapeutic use. The net result was beneficial to the therapeutic self-care industry. Consumer confidence in the quality and benefits of OTC medicines rose. The industry understood the rules of the road, and competition was based on fair play. An effective enforcement program by FDA has resulted in few occurrences of rogue firms “tainting the well” of consumer confidence with sub-standard products.

Today, FDA – and the Federal Trade Commission (FTC) – have substantial statutory authority to regulate dietary supplements consistent with DSHEA.

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** The Consumer Healthcare Products Association (CHPA), formerly known as the Nonprescription Drug Manufacturers Association (NDMA), is the 118-year-old trade organization representing the manufacturers and distributors of national and store brand dietary supplements and nonprescription medicines. CHPA’s membership includes over 200 companies involved in the manufacture and distribution of these self-care products and their affiliated services (e.g., raw material suppliers, research testing companies, contract manufacturing companies, advertising agencies, etc.).

The Food and Drug Administration has the power to:

1. Stop any company from selling a dietary supplement that is toxic or unsanitary [see Food Drug and Cosmetic (FDC) Act, Section 402(a)];
2. Stop the sale of a dietary supplement that has false or unsubstantiated claims [see FDC Act, Section 403(r)(6)];
3. Take action against dietary supplements that pose “a significant unreasonable risk of illness or injury” [see FDC Act, Section 402(f)];
4. Stop any company making a claim that a product cures or treats a disease [see FDC Act, Section 201(g)];
5. Stop a new dietary ingredient from being marketed if FDA does not receive enough safety data in advance (see FDC Act, Section 413);
6. Require dietary supplements to meet strict manufacturing requirements (Good Manufacturing Practices), including potency, cleanliness, and stability [see FDC Act, Section 402(g)].

The Federal Trade Commission has the power to:

1. Enforce laws outlawing “unfair or deceptive acts or practices” to ensure consumers get accurate information about dietary supplements, so they can make informed decisions about these products [see Federal Trade Commission (FTC) Act, Section 5];
 - An unfair trade practice is one that: causes or is likely to cause substantial injury to consumers; is not reasonably avoidable by consumers themselves; and not outweighed by countervailing benefits to consumers or competition (see FTC’s Deception Policy Statement and Advertising Substantiation Policy Statement);
2. Challenge and stop advertising that is not adequately substantiated (see FTC’s Deception Policy Statement and Advertising Substantiation Policy Statement);
3. Investigate complaints or questionable trade practices;
 - FTC can investigate either informally or formally, where it has strong compulsory investigative authority, including the power to require a respondent to produce documents, give testimony, or answer written questions [see FTC Act, Sections 6(a and b) and 9];
4. Following its own investigation, negotiate a consent order or proceed through an FTC adjudication resulting in a cease and desist order, which can be quite broad in its scope (see FTC Act, Section 5);

5. Seek preliminary and permanent injunctions to stop false advertisements or other violations of the FTC Act. [see FTC Act, Section 12 and 13];
6. Seek civil penalties for violations of trade regulation rules [FTC Act, Section 5] or of cease and desist orders (see FTC Act Section 5).

This is not to say stiffer regulations that are counter to the intent of DSHEA should be sought for dietary supplements. DSHEA is a good law. By its very title -- the Dietary Supplement and Health Education Act -- it was the clear intent of Congress in enacting it that consumers should be provided with products, information, and education that would help promote health and prevent disease (through maintenance of healthy aspects of the structure and function of their bodies). In fact, Congress notes in the "findings" section of the Act, "consumers should be empowered to make choices about preventive health care programs based on data from scientific studies of health benefits related to particular dietary supplements." In so doing, Congress did not diminish the enforcement authority of either FDA or FTC. In fact, FDA was further empowered, for example, to issue Good Manufacturing Practices for DSHEA, something the industry has strongly supported. DSHEA therefore has important implications for the improvement of health for all Americans, through the education imparted by more informative labeling and advertising and through the resultant expanded use of quality dietary supplements for health maintenance and promotion and disease risk reduction.

Importantly, FDA has acknowledged the findings of Congress and that its current enforcement powers are adequate. In her statement to Congress on March 25, 1999, FDA Commissioner Jane E. Henney, M.D. stated:

"DSHEA amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) to define the term "dietary supplement" and establish a regulatory framework for dietary supplements. In doing so, Congress made 15 significant findings that emphasize the importance of diet and nutrition, including dietary supplement use, in promoting health and reducing the risk of disease. FDA acknowledges these findings."²

Dr. Henney also stated at that time: "FDA has tools at its disposal to take enforcement actions against dietary supplements found to have safety, labeling, or other violations of the FD&C Act, as amended by DSHEA."²

What is needed therefore is an effective, yet reasonable regulatory approach to the enforcement of DSHEA. For example, on advertising and promotional activities, FTC has issued a special guidance to the dietary supplement industry which is based on the FTC Act and long-standing policies defining the boundaries of the advertising and promotion of consumer products.⁵ CHPA has adopted a statement supporting FTC's role in this area.⁶

The FDA has not been inactive. Over the past 5 years, the FDA has published almost 100 documents in the *Federal Register* relating to dietary supplement labeling, FDA pro-

gram activities, etc. Yet recently, a number of groups have come forward suggesting special quality seals for qualifying dietary supplements, including Consumerlab.com and the American Nutraceutical Association. This suggests a lack of an effective enforcement program by FDA, since such government activity would presumably be the incentive needed for all companies to ensure their products meet reasonable standards of identity and quality.

However, over the next year to year-and-a half, we can expect the level of FDA's regulatory presence to increase through the issuance of a Final Rule on structure/function claims, an overall strategy for dietary supplements, a list of program priorities for the Center for Food Safety and Applied Nutrition, proposed GMPs (and presumably with it a more visible inspections program by FDA), and better management of adverse experience reports. Whether that is accompanied by an effective and reasonable FDA enforcement presence consistent with DSHEA remains to be seen. FTC can be expected to continue to monitor print and electronic advertising and promotion, bringing cases as needed in the context of its guidance to industry.⁷ Recently, Ms. Jodie Bernstein, Director of the FTC Bureau of Consumer Protection, pledged FTC's commitment, "... FTC must and will continue to maintain an enforcement presence here, giving priority to cases that present serious safety considerations or prey on the very sick and especially vulnerable consumer."⁸

To the informed regulatory scientist, product development expert, and legal expert within dietary supplement companies committed to excellence, this should not be unwelcome news, since consumer confidence is the ultimate goal. To companies that have not been paying attention to the winds of regulatory change, this should be a wake-up call to ensure your company is meeting the new expectations of the marketplace. To health professionals and consumers, these developments should be encouraging.

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Nutraceuticals for Your Pet

**DSHEA, which governs human dietary supplements,
does not apply to animals**

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The use of dietary supplements by humans is booming across the country and, slowly, so is the awareness of some of the problems relating to the safety and efficacy of these products. Dietary supplements are commonly referred to as nutraceuticals, functional foods, or food supplements. But did you know when Congress passed, and the President signed into law the Dietary Supplement Health & Education Act (“DSHEA”) in 1994, that this Act did not to cover dietary supplement products intended for animals?

How are dietary/food supplements, nutraceuticals, and/or functional foods classified and regulated for animals, and how does this affect products that you may be administering to your pet? That is a complex and interesting question and one we will attempt to answer, or at least bring out into the open to raise consumer awareness. Please remember, this is an evolving regulatory area, and this issue is just beginning to be addressed by state and federal regulatory officials with the help of the industry and its various trade associations.

Regardless of the regulatory issues with animal dietary supplements, the same problems exist for both animal and human products. Are these products safe and effective and how can consumers be sure? This article is not intended to answer these questions outright, but to educate consumers as to what the current situation is for animal dietary supplement products and what consumers can do to take an active role to help ensure that dietary supplement products intended for your pets are safe and effective.

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By way of background, the Center for Veterinary Medicine (CVM), the federal agency at the Food & Drug Administration that regulates health products for animals, has stated on the record that DSHEA does not apply to products intended for use with animals.¹ So how are these products regulated for your pet?

DSHEA

Since readers of *JANA* are well-educated by previous articles about DSHEA, we will not go into a formal detailed analysis as to the role of DSHEA. However, to better understand how this affects your pet’s products, you need to know some parts of DSHEA. Essentially, DSHEA defines and regulates a specific category of products under the classification of food. Dietary supplements are defined under DSHEA as follows:

- is a product (other than tobacco) that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients.
- is intended for ingestion in pill, capsule, tablet, or liquid form.
- is not represented for use as a conventional food or as the sole item of a meal or diet.
- is labeled as a “dietary supplement”.
- includes products such as an approved new drug, certified antibiotic, or licensed biologic that was marketed as a dietary supplement or food before approval, certification, or license (unless the Secretary of Health and Human Services waives this provision).

DSHEA sets up certain regulations and requires/allows FDA, through its Center for Foods and Applied Nutrition (CFSAN), to develop rules and guidelines for dietary supplements relating to safety, literature, nutritional support statements (structure and function claims), ingredient and nutrition labeling, new dietary ingredients, and good manufacturing practices (GMPs).

DSHEA was established on the principle that consumers should be permitted to make informed choices about safe dietary supplements intended to augment their daily diets and provide health benefits. One thing DSHEA does not do is to provide for pre-market approval by the FDA for these products. Currently, not all of DSHEA has been fully implemented, such as the establishment of GMP regulations regarding the preparation, packing, and holding of dietary supplements to ensure their safety. There are several ongoing court cases and/or disputes relating to health claims² and what constitutes a conventional food, dietary supplement, or drug.³

There also exists confusion and disagreement relating to what is a structure and function claim, implied disease claim, express disease claim, and the definition of disease. To confuse matters more, none of this legally affects the products for pets since DSHEA does not apply to products intended for use with animals.

ANIMAL DIETARY SUPPLEMENTS LEGAL STATUS

To understand how animal dietary supplements are regulated, you must first understand what legal terms and definitions are in existence for these products. Under current federal regulations these will either be classed as foods, food additives, or drugs. The legal definitions are found under the Federal Food, Drug & Cosmetic Act (FFDCA), Section 201.⁴

Most animal dietary supplements come in a capsule, tablet, powder, or liquid, however many pet food manufacturers are incorporating these dietary ingredients into their pet foods on the basis that these ingredients are Generally Recognized As Safe ("GRAS") and are not classified as food additives.⁴

For the purposes of this article, we will not discuss products intended for food-producing animals (livestock) but will focus on products intended for companion animals (e.g., dogs, cats, and non-food horses). Resource limitations make it difficult for CVM to establish any regulatory framework to oversee dietary supplements for animals. Congressional modification to DSHEA would be time-consuming and is believed by many regulatory officials as unnecessary.⁵ Recently, the CVM has indicated support for the Association of American Feed Control Officials (AAFCO) to develop guidelines for veterinary dietary supplements. AAFCO, whose roots go back to 1909, was set

up to establish an association through which officials of any local, state, or federal government charged with the responsibility of enforcing laws regulating the production, labeling, distribution, or sale of animal feeds may unite to explore problems and promote uniformity in administering and enforcing laws affecting the animal feed industry.⁶

Continued open and effective communication and cooperation between the CVM and AAFCO is essential in order to develop a guideline or regulatory framework which supports the principles of DSHEA within the practicality of the animal feed and pet food industry. Traditionally, animal feeds including pet foods have been mostly regulated by the states that follow model guidelines set up through AAFCO and Federal Regulations.

AAFCO's Feed Labeling Committee, subcommittee on Nutritional Health Claims, and Nutrient Functional Claims met several times over the past several years to receive comments and information on the issue of animal dietary supplements because they recognized the need for regulatory support and consistent regulatory interpretation. This resulted in a meeting between representatives of the AAFCO Board of Directors and officials from the CVM.

This meeting concluded with a pledge of support from the CVM to participate with the AAFCO Board of Directors and in the appointment of the Nutraceutical Regulatory Advisory Panel (NRAP) at the Annual Meeting in Bismarck, North Dakota in August of 1998. The NRAP consulted with industry and trade associations over the past 1-1/2 years and presented their Final Report to the AAFCO Board of Directors at the Annual Meeting in Omaha, Nebraska in August 1999. Several proposals came out of that meeting, most of which were accepted by the Membership of the Board of Directors.⁵ Most importantly, two task forces were set up in order to prepare and implement guidelines: (1) The Novel Feed Ingredients Task Force; and (2) Botanical and Herbs Task Force.

It is the position of AAFCO and the NRAP that the current legal terms of food, food additive, and drug have been defined and exist in current laws and regulations. These do not need further clarification for dietary supplements for animals even though Congress recognized this need for dietary supplements for humans.⁵ However, AAFCO (including CVM) recognizes a need to allow these products to be marketed to animals if they pose no significant health risk and are safe for the intended species.⁵

In order to make these definitions fit, one of the NRAP recommendations is that the AAFCO definition of the term "nutrient" be reviewed and considered more broadly to include substances other than "essential nutrients" such as various types of orally ingested substances that provide natural components of the body and/or offers nutrients that affect physiological well-being of the animals. A broader definition of nutrient may be necessary if dietary supple-

ments eventually fall under the AAFCO ingredient definition process.

Essentially all ingredients fed to animals to meet their nutritional needs are defined under a joint AAFCO and CVM ingredient definitions procedure. Undefined ingredients may be prohibited by the State Feed Control Officials unless they are considered an approved food additive or GRAS under federal law. However, individual states and even CVM have recognized the Ingredients Definitions that are listed in the Official Publication even though these ingredients may not be approved food additives or GRAS.

There is also the possibility for manufacturers to utilize the new GRAS notification process under Federal law which is designed to streamline and make the former GRAS affirmation more user friendly.⁷ This is a rapidly evolving process that will hopefully provide an appropriate regulatory framework for uniform allowance (not to be confused with approval) by the States and CVM of dietary supplement to animals in the near future.

TRADITIONAL TERMS FOR COMPANION ANIMALS

There is a recognized need to allow certain dietary supplements for animals when these products can demonstrate they are safe for animal use. Certain structure and function claims about their utility should be permitted so that consumers and vets can make informed choices about these products for their pets. However, until this is all worked out, consumers and vets must take an active role to educate themselves about these products and ask the right questions to make sure that they are giving their pets/patients safe and effective products.

Companion animals are routinely fed complete, balanced diets and are regulated by different rules than are conventional foods for human consumption.⁸ There are also rules for labeling "treats and chews" offered on an occasional basis and not fed as the mainstay of the pet's diet. In addition, there are special rules relating to weight control products, dental products, skin, and coat products and veterinary medical foods. For purposes of this article, we will only discuss dietary supplement ingredients used in pet foods or in supplement form (pill, tablet, or powder form not put in a complete and balanced pet food or feed).

Nutritional supplements for pets have been available for many years, however these traditionally are products that provide a source of a recognized essential nutrient, such as calcium or vitamin A and are intended to augment and ensure nutritional completeness of the diet and follow the same rules as other pet foods.⁹ If it claims to be a vitamin or mineral supplement, the label must bear guarantees (claimed amounts) for each vitamin or mineral in the product. The suggestion spoken of earlier, to consider a broad-

er definition of nutrient, may be helpful because of the traditional use of these products for pets.

EDUCATION AND AWARENESS ARE KEY

Questions need to be asked by the consumer, veterinarians, and staff of the magazines running ads for animal dietary supplement products similar as those asked of manufacturers and/or distributors of human dietary supplements. A leading consumer magazine, which has always insisted the claims made for advertised products be truthful, non-misleading, and substantiated by valid scientific data, required manufacturers of dietary supplements for humans to substantiate their health-benefit claims with placebo-controlled, double-blind studies published in peer-reviewed journals.¹⁰ In addition, they required manufacturers to provide proof that their products contain the same ingredients, in the labeled quantity, as listed on the labels.¹⁰ Currently only three dietary supplement manufacturers were able to meet that criteria.¹⁰ Some trade and consumer groups across the nation are beginning to request similar criteria from the manufacturers and distributors of dietary supplements.

A recent article in the *Compendium on Continuing Education for the Practicing Veterinarian*[®] September 1999 issue (Volume 21(9)),¹¹ suggested that "...when evaluating nondrug oral products, veterinarians must base their selection decisions on their knowledge of the compound's safety and efficacy and the manufacturer's reputation. One standard by which veterinarians can evaluate a manufacturer is whether the product has been subjected to independent laboratory analysis and clinical trials and has been made using good manufacturing practices."

In addition, the article states that "...veterinarians should also study labels to ensure the exact amounts of active ingredients are listed and the labels are easy to understand..." and "Veterinarians should request a copy of the analysis of the finished product – not the raw material – from the manufacturer because a raw product does not determine whether the product was properly manufactured."

Currently, GMPs are not required for dietary supplements for companion animals or for pet food. GMPs include quality assurance steps to ensure that there is not cross-contamination from other products, there is batch-to-batch consistency, and that the product meets the label claim. The GMPs help ensure safe products reach the consumers.

Do not be afraid to call the manufacturer and ask for answers to these questions and, if possible, have them send you written documentation. Consult your veterinarian on these matters as well. It pays to be an informed and educated consumer because, in this industry, it is buyer beware.

CONCLUSION

So what does the future hold? Hopefully, some recommendations are forthcoming from the Novel Feed Ingredients Task Force to establish some regulatory guidelines. The AAFCO Herbal and Botanicals Task Force is also in the infancy stages and it is uncertain how these products will be handled. The AAFCO manufacturing committee is actually starting the process to require some basic GMPs for feeds that will likely include dietary supplement products, and dietary supplement ingredients incorporated into pet foods.

Until that time, it is highly recommended that the consumer be aware that these products may not be closely monitored and have not had pre-market approval by Federal or State regulatory officials. Therefore, the consumer must carefully educate themselves when making choices about the dietary supplement products they purchase for their pets.

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Food Additive: Defined by FFDCA Section 201(s) as “any substance the intended use of which results or may reasonably be expected to result, directly, or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or in the case of a substance used in food prior to January 1, 1958 through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use.” There are exemptions to this definition relating to pesticides, color, additives, substances not granted approval before the enactment of the Poultry Products Inspection Act or the Meat Inspection Act of March 4, 1907 or a new animal drug.

Drug: Defined by FFDCA Section 201(g)(1) as “(A) article recognized in the official United States

Pharmacopeia, official Homeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of many or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C); but does not include devices or their components, parts, or accessories.”

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Chronic Fatigue Syndrome An Overview and Update

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"Imagine waking up every morning exhausted and feeling like you have the flu. Imagine this feeling dragging on for months, even years, undermining your career and personal life. Then imagine seeking help and being told, 'This isn't a real illness. You're just depressed!'"

- 41 year old patient with CFS

"I've been on call all weekend and I have 26 patients scheduled today. I don't have time for hypochondriacs who need 90-minute visits. Who doesn't feel tired and achy all the time?"

- 52 year old internist

Most clinicians have heard similar laments from patients and colleagues alike. There is no doubt that chronic fatigue syndrome and fibromyalgia are a source of tremendous frustration for everyone involved. Patients feel miserable and frequently receive little credence or empathy. Practitioners feel torn between a long list of symptoms and a stack of "normal" test results. Researchers feel perplexed by growing reports of subtle abnormalities in measures of immune system, endocrine system, and central nervous system function. Despite nearly two decades of confusion and controversy, chronic fatigue syndrome and fibromyalgia are indeed "real". Many clinicians and researchers believe these two disorders represent different points along the same spectrum of illness. They may well be correct. For the sake of clarity, however, this overview and update will consider the diagnostic criteria, possible pathophysiology, and comprehensive treatment strategies for CFS. Fibromyalgia will be reviewed in a later article.

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CHRONIC FATIGUE SYNDROME:

Demographics and Diagnostic Criteria

Chronic fatigue syndrome (CFS) is a debilitating condition first defined in 1988,¹ although descriptions of a similar syndrome can be traced back hundreds of years in medical writings and literature. The exact prevalence is uncertain since there is no single diagnostic test to date. CFS has no pathognomonic findings on physical exam but is instead a heterogeneous disorder with apparently multifactorial causes. There is no doubt that CFS often follows an acute infectious illness,^{2,3,4} although some patients have no clear precipitating infection. Several studies point to a possible disruption of the hypothalamic-adrenal-pituitary axis and neurally mediated postural hypotension.^{5,6,7} Neurotransmitter depletion, cytokine abnormalities, environmental factors, psychosocial stress, and genetic predisposition may all play a role in various subsets of patients with CFS.

Regardless of the etiology, CFS affects women more often than men, and its prevalence seems to vary according to geographic location and even occupation. An apparently higher prevalence has been noted by some clinicians in day care workers, schoolteachers, airline personnel, and health care workers. But, clearcut research supporting this observation is limited. Prevalence rates vary from 75 per 100,000 to 230 per 100,000 in community surveys that excluded individuals previously found to be clinically depressed.^{8,9} A survey of Chicago-based nurses revealed a current CFS prevalence rate of 1,088 per 100,000 and a lifetime prevalence of 1,265 per 100,000.¹⁰ It may be that nurses represent a high risk group as the result of the stresses of shift work and increased exposure to viruses..

While CFS is not unknown in children and teenagers, the majority of patients are in the 30 to 50 year old range. Initially, the syndrome was thought to affect mostly well-educated, upper middle class white women, but it is now clear that CFS affects people from all ethnic backgrounds, income categories, and educational levels.¹¹

Fatigue is undeniably one of the most common subjective complaints encountered in clinical practice. Virtually any illness from a cold to cancer is capable of causing mild to incapacitating fatigue. In 1994, the consensus criteria for the diagnosis of CFS was updated¹² from that used in 1988. Presently, the diagnosis of CFS requires:

1. Clinically unexplained fatigue that has persisted for 6 months or more. The fatigue must not be related to exertion nor relieved by rest, and results in significant reductions in social, personal, educational, and professional activity levels.
2. Four or more of the following symptoms on a concurrent and persistent basis:
 - Impaired concentration or short term memory
 - Sore throat
 - Tender cervical or axillary lymph nodes
 - Myalgias
 - Arthralgias without arthritis
 - Headaches new in character, severity, or pattern
 - Unrefreshing sleep
 - Post-exertional fatigue that lasts more than 24 hours

The neuropsychologic symptoms associated with CFS are often a source of great distress to patients. Unfortunately, they have also given rise to considerable skepticism among many physicians since similar symptoms are characteristic of major depression. Pronounced fatigue, mood changes, psychomotor slowing, lethargy, cognitive impairment, and sleep disturbances are seen in clinical depression and CFS. However, such symptoms are also frequently observed in patients with Parkinson's disease, multiple sclerosis, systemic lupus erythematosus, severe infectious diseases, and neoplastic processes. Dismissing a constellation of neuropsychologic symptoms as "just depression" is inappropriate. A huge body of evidence gathered over the past twenty years has demonstrated that depression has a neurochemical basis. Disturbances in neurotransmitter levels (e.g. serotonin, dopamine) may be triggered or aggravated by a long list of negative or traumatic experiences, but that in no way lessens the neurophysiologic consequences. It is sobering to reflect upon the fact that before the advent of MRI scans, testing for oligoclonal bands, or antinuclear antibodies, many patients with MS and SLE struggled for years without a diagnosis other than depression or hypochondriasis. The development of a definitive test changes our approach to an illness. The absence of a definitive test, however, has never lessened a patient's suffering. On the contrary, such diagnostic limitations may add insult to injury when a patient's misery is dismissed as imaginary.

CFS: A Sensible Approach to Diagnostic Studies

Once a careful history has been obtained and a thor-

ough physical exam has been completed, appropriate laboratory tests are warranted to exclude other diseases. A reasonable work-up includes a complete blood count, chemistry profile, thyroid function studies, urinalysis, liver enzymes, erythrocyte sedimentation rate, rheumatoid factor, and antinuclear antibodies. Based on a patient's history and risk factors, testing for HIV, Lyme disease, and/or TB may be indicated. Routine use of MRI scanning is unnecessary. Some clinical investigators recommend checking serum carnitine levels since a subset of CFS patients have been found to respond to supplementation with L-carnitine.¹³ Periodic reassessment of these laboratory tests is advisable since occasional patients thought to have CFS later proved to have other illnesses.

CFS: Current Views of Pathophysiology

Clinical and research experience over the last 15 years has demonstrated that CFS is a heterogeneous disorder with various subsets of patients and multifactorial causes. Hence the controversy. A single unifying agent or etiology would be easier to accept, diagnose, and treat. So far, evidence exists for abnormalities in natural killer cell numbers and function, cytokine production, reduced functional B vitamin status, hypothalamic-pituitary-adrenal axis function, blood flow to the brainstem, cortisol levels, and neurotransmitter synthesis. An underlying genetic predisposition, various occupational and environmental exposures, and sensitivities along with psycho-social stress responses may all play a role in the disorder. Presumably, several of these aberrations may trigger a pathophysiologic cascade leading to the CFS symptom complex.

The immunologic abnormalities demonstrated in some studies of CFS patients are subtle, insidious, and complex. They share some of the features of immune dysfunction seen in medical students under examination stress and caregivers of patients with Alzheimer's disease.¹⁴ Evidence exists for an increase in suppressor T-cell function,¹⁵ down-regulation of natural killer cell lysis,^{16,17} changes in cytokine synthesis by peripheral blood lymphocytes *in vitro*,^{18,19} and low NKH1+T3- cells resulting in an elevated NKH1+T3+:NKH1+ratio.²⁰ Such intriguing and important studies are unavailable to most practicing clinicians.

A somewhat more practical area of study has explored the connection between low blood pressure and fatigue.²¹ In one study of 10,314 British civil servants, the lowest quartile of systolic blood pressure was associated with the highest rates of somatic complaints, dizziness, and fatigue.²² Research conducted at Johns Hopkins University School of Medicine has found evidence of neurally mediated hypotension (NMH) in 77% of some 600 CFS patients.^{23,24} NMH is generally seen in women under 50 and may occur after an acute infection. Symptoms related to NMH often worsen after physical exertion, hot baths or showers, or prolonged

standing, especially in a warm environment. Emotional stress, pain, and a variety of drugs (vasodilators, diuretics, phenothiazines and tricyclic antidepressants) may also aggravate orthostatic intolerance. Periods of prolonged inactivity or bedrest, in addition to causing cardiopulmonary deconditioning, may decrease plasma volume and red blood cell mass. By reducing potential triggers of NMH and expanding blood volume with sodium and fluid intake, some CFS patients may manage to alleviate certain symptoms.

Another intriguing line of research into the pathophysiology of CFS is investigating subtle abnormalities in the reticular activating system (RAS) and brainstem. Although it is not a consistent finding, several MRI studies have shown small, discreet, patchy lesions in the brainstem and subcortical areas of patients with CFS.^{25, 26} Such lesions are difficult to interpret and appear to be nonspecific. Potentially more significant findings have been demonstrated with PET scanning (positron emission tomography). One study compared results of PET imaging in patients with CFS to those with major depression.²⁷ CFS patients showed significant hypometabolism of the right mediofrontal cortex and brain stem. Patients with depression had impaired glucose metabolism in the frontal cortex. Similar patterns have been reported in a study using SPECT (single photon emission computed tomography).²⁸

Comparable areas of regional blood flow reduction have been noted in patients with multiple sclerosis and post-polio fatigue syndrome.²⁹ Further study into this area should help identify a more distinct therapeutic target for CFS.

Lethargy, fatigue, and depressed mood are common features of many disorders including Cushing's disease, hypothyroidism, and seasonal affective disorder. A deficiency of corticotropin-releasing hormone (CRH) has been well-documented in these conditions^{30, 31} and may also occur in a subset of patients with CFS. Compared to healthy individuals, patients with CFS appear to have a mild glucocorticoid deficiency.³² Unfortunately, long term treatment with steroids is fraught with far too many complications to be considered clinically. However, the theory that hypofunctioning CRH neurons seen in different illnesses represents a final common biologic pathway causing similar symptom complexes is intriguing.

In a somewhat similar vein is the observation that many patients with HIV disease and Lyme disease, who frequently have significant fatigue and depressive symptoms, have abnormal CNS metabolism of serotonin and tryptophan. Decreased tryptophan levels, along with increased tryptophan catabolites (kynurenic acid and quinolinic acid) have been reported in these patients.³³ *In vitro* studies have shown that interferon (gamma) precipitates the degradation of tryptophan.³⁴ It has been theorized that activation of cell-mediated immunity in CFS increases levels of interferon- γ which then interferes with tryptophan metabolism.³⁵

Ultimately, serotonin levels fall and depressive symptoms, pain, and fatigue result. This theory has practical appeal in the treatment of CFS since selective serotonin reuptake inhibitors (SSRIs) in low doses are safe. These drugs are generally well-tolerated even in CFS patients who tend to be sensitive to medications with CNS effects.

Post-exertional fatigue has certainly been one of the most demoralizing symptoms for patients with CFS. It has also confounded clinicians and researchers. It seems clear that many patients with CFS are capable of aerobic activity, yet experience such malaise afterwards that even nominal activity is perceived as exhausting. A variety of subtle intramuscular abnormalities have been identified in sporadic cases (increased acidosis,³⁶ abnormal jitter on EMGs,³⁷ depressed muscle mitochondrial respiration³⁸ and abnormal mitochondrial structure).^{39, 40} One particularly interesting study examined the effect of exhaustive exercise on cognitive performance of patients with CFS. Using treadmill testing and a battery of 4 cognitive tests, CFS patients were compared to healthy controls. No differences in cognitive function were observed before exercise. However, after exhausting exercise, CFS subjects showed slower cognitive processing compared to healthy persons.⁴¹ Given the nearly universal experience of post-exertional fatigue and malaise in patients with CFS (and fibromyalgia), further study into this aspect of the illness is warranted.

Another possible etiology of the symptoms described by CFS patients is an allergic process or environmental chemical intolerance. Allergic reactions seem to occur with greater frequency in CFS patients than in the general population. Chemical intolerance is found in 4-6% of the general population but occurs in 20-47% of individuals with CFS.⁴² Also known as multiple chemical sensitivity (MCS) or sick building syndrome, this phenomenon may amplify a number of endogenous responses in the immune system, endocrine system, and/or CNS that produce symptoms of CFS.

For now, it seems unlikely that a single, discrete cause for CFS will be established. It may be that susceptible individuals develop CFS as the endpoint of a pathophysiologic cascade of events. This cascade may be triggered by an apparently wide array of physiologic stresses affecting the immune system, CNS, endocrine system, or cellular metabolism. Two things do seem certain. CFS is not imaginary and it is not going away. Finding a way to help patients feel better and regain function seems more worthwhile than debating the "reality" of their suffering.

CFS: Comprehensive Treatment Strategies

At the present time, there is no "magic bullet" to treat CFS. The most effective treatment addresses specific symptoms such as myalgias, arthralgias, headaches, and sleep disturbance, while incorporating lifestyle practices to improve stamina, boost nutrition, and manage stress.

Balanced nutrition with fresh, high quality foods is a key component of the recovery process. And yet, for a patient suffering debilitating fatigue, achieving good nutrition is easier said than done. Grocery shopping and food preparation can be exhausting and few patients with CFS have a personal shopper or cook. Practical suggestions include drinking fresh juices, snacking on nuts (essential fatty acids, protein, and magnesium), cottage cheese or yogurt, replacing iceberg lettuce with fresh spinach (natural carotenoids, iron, B₁₂, vitamin C), and choosing whole grain breads and pasta. Foods such as oatmeal and eggs (protein, B vitamins) are easy to prepare and digest. Eggs are probably the best dietary source of choline (consider choline acetyltransferase in cognitive function) and also contain the essential fatty acid DHA (docosahexanoic acid). DHA is the most abundant fatty acid in the brain and is critical for normal neurocognitive function. Deficiencies are associated with memory loss, depression, and visual impairment. Cold water fish (herring, halibut, salmon, tuna, mackerel) are excellent sources of DHA as well.

Neither assessing a patient with CFS nor providing guidance about therapy can be accomplished in a 7-minute office visit. An approach comparable to that used in cardiac or stroke rehabilitation programs is most likely to meet with some success. Confronting a patient's belief system about their illness is rarely helpful. A practitioner who is willing to listen and accept the patient's illness at face value is probably as important in the recovery process as any specific intervention.

Overall, treatment which includes mild, graduated activity, low dose SSRIs, cognitive behavioral therapy, and correction of sleep disruption has been most effective.^{43,44} However, patients and clinicians alike should understand that a realistic treatment goal is reducing malaise and fatigue, and improving functional ability, not achieving a "cure."

A crucial intervention in the treatment of CFS is restoration of normal sleep structure. Unrefreshing sleep is one of the hallmarks of CFS and many patients fail to enter deep, stage IV sleep. Experience in clinical settings has shown zolpidem (Ambien) to be helpful inducing sleep onset and reducing nighttime awakenings without disrupting normal sleep structure. The half-life of zolpidem is quite short, reducing the chance of daytime drowsiness. Some patients with CFS are extremely sensitive to drugs with any CNS effect, and doses as low as 2.5 mg may be helpful in breaking patterns of insomnia or disturbed sleep. Clearly, healthy sleep habits such as retiring and rising at the same time each day, avoiding caffeine, nicotine, and alcohol, maintaining a comfortably cool ambient temperature and incorporating relaxing bedtime rituals (warm bath, meditation, music therapy, etc) improve the likelihood of obtaining restful sleep.

The use of low dose antidepressants has been helpful in some patients with CFS. Curiously, the doses used are

much lower than those needed to treat clinical depression. Tricyclic antidepressants such as amitriptyline are usually poorly tolerated in CFS. The SSRIs such as fluoxetine (Prozac) and sertraline (Zoloft), again in low doses, seem better tolerated. The reversible monoamine oxidase inhibitor, moclobemide has been shown to reduce fatigue, anxiety, depression and somatic amplification in CFS patients with co-morbid major depression.⁴⁵

Concern about abnormal mitochondrial function in patients with CFS has focused attention on therapy with supplements such as carnitine and coenzyme Q₁₀. Low serum levels of carnitine have been reported in a subset of patients with CFS.⁴⁶ In one study, CFS patients with low to low-normal carnitine levels improved in 12 of 18 parameters of mental and physical fatigue after 4 to 8 weeks of L-carnitine supplementation.⁴⁷ The dose was 1 gram three times a day and side effects were nominal.

A growing body of medical research lends support to the use of coenzyme Q₁₀ in the treatment of patients with hypertension, congestive heart failure, and cardiomyopathy.^{48,49} Coenzyme Q₁₀ levels in the body peak during the third decade and gradually decline. A naturally synthesized vitamin-like substance, Coenzyme Q₁₀'s most clearly defined role is in ATP production in the mitochondrial respiratory chain. It also functions as an antioxidant.⁵⁰ Adequate studies of coenzyme Q₁₀ in the CFS setting are lacking. However, an interesting trial of coenzyme Q₁₀ in patients with muscular dystrophy or neurogenic atrophy demonstrated improvement in cardiac output, stroke volume, and measures of subjective physical well being.⁵¹ Since coenzyme Q₁₀ is a readily available, safe nutritional supplement, a two to three month trial in patients with CFS may be a reasonable consideration.

Another substance involved in the generation of ATP is nicotinamide adenine dinucleotide (NADH). Research conducted at Georgetown University School of Medicine examined the effect of the stabilized oral absorbable form of NADH (ENADA) in a randomized, double-blind, placebo-controlled crossover study in patients with CFS. After 4 weeks of treatment with 10 mg per day of NADH, 31% of patients had a beneficial response compared to an 8% response rate with placebo.⁵² An open-label study in a larger cohort of patients is now underway.

Isolated studies have reported symptomatic improvement in CFS patients treated with magnesium,⁵³ essential fatty acids (evening primrose oil, and fish oil),⁵⁴ and high dose intravenous immunoglobulin (IVIG).⁵⁵ So far, other studies have not been able to produce similar results.

The distinct subset of CFS patients with neurally mediated hypotension (NMH) may respond well to increased fluid and sodium intake and avoidance of trigger situations (prolonged standing, warm environments, hot

baths, and showers).⁵⁶ Additional treatment with low dose beta blockers and mineralo-corticoids (Florinef) may be useful in carefully selected patients, but these measures do increase the risk of adverse effects.

A useful nonpharmacologic treatment for CFS (and other chronic conditions) is cognitive behavioral therapy (CBT). This approach is based on the idea that a patient's thinking and beliefs about their illness (cognitions) and coping skills may help or hinder the recovery process. CBT entails a fairly complicated set of techniques employed in 10 to 20 one hour sessions with a therapist (in individual and/or group settings). The goal is to help patients assess their current illness-related beliefs and behaviors, reformulate less catastrophic interpretations of their symptoms, and develop more constructive personal, occupational, and social coping skills. There is evidence to support the efficacy of CBT in patients with CFS.⁵⁷ Motivation, compliance, and availability of specially trained therapists is key.

Another potentially useful, virtually risk-free treatment for certain CFS patients is light therapy. A subgroup of patients with CFS shows seasonal variations in symptoms comparable to that observed in persons with seasonal affective disorder.⁵⁸ Light therapy has been used successfully to treat a number of these patients.⁵⁹ Homebound CFS patients may be chronically underexposed to light, augmenting neurovegetative and depressive symptoms. Further study in this area may confirm the usefulness of year-round light therapy for patients with a number of chronic conditions.

There is little doubt that individuals with CFS achieve the best results when engaged in a multifaceted ongoing treatment program. A comprehensive Canadian program evaluated 51 patients with CFS.⁶⁰ The treatment was specifically tailored to individuals and included 7 components:

1. Structured physical exercise and activity
2. Sleep management strategies
3. Careful activity management
4. Reduction of stimulant intake and symptomatic medications
5. Cognitive behavioral therapy
6. Family participation
7. Efforts to establish specific vocational and avocational goals

Employers were also encouraged to facilitate a gradual, time-targeted return to work. Thirty-one of 51 treated patients returned to employment and 14 functioned at an equivalent level, 6 patients remained relatively disabled. Few patients in the untreated group showed improvement.

Research, clinical experience, and common sense tell us that no chronic illness exists in a psycho-social vacuum. Hundreds of studies confirm the relationship between stress and illnesses ranging from colds to cancer. The beneficial effects of positive emotional states, humor and laughter are

well-documented from a physiologic perspective.^{61,62} Similarly beneficial effects have been observed in response to meditation.^{63,64} And, mounting scientific evidence indicates that religious practice, faith, and prayer play an important role in the healing process and serve as practical coping strategies for significant numbers of patients.^{65,66}

A variety of complementary therapies may be helpful in the treatment of CFS, at least in terms of symptomatic relief. Acupuncture and massage may augment traditional therapy for myalgias, arthralgias, and headache. Focused, low impact exercise such as yoga and tai chi can be modified to accommodate the individual needs and limitations of many CFS patients. And certain herbal remedies such as ginseng, ginkgo biloba, and St John's wort may be worth trying for fatigue, cognitive problems, and depression respectively. As is the case with any medical intervention, appropriate cautions and contraindications must be observed.

CONCLUSION

CFS remains a challenge for patients, family members, and clinicians alike. It forces us to confront the pitfalls of physiologic reductionism both in research and primary care practice. As noted researcher Dr. Mark Demitrack wrote, "As an illness, CFS straddles the traditional Western dualism of mind and refuses to fit neatly into either of the separate domains of internal medicine or psychiatry"⁶⁷. Precisely. Unless science discovers that "magic bullet" for CFS (which seems unlikely), clinicians and patients will need to work together in a comprehensive integrative approach that seeks to restore well-being and function.

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Phytonutrients and Metabolic Stimulants as Protection Against Neurodegeneration and Excitotoxicity

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There is ample evidence that one of the central causes of neurodegenerative disorders and other brain insults is excitotoxicity. The list of such disorders includes Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, strokes, brain trauma, viral, mycoplasmal and rickettsial infections of the central nervous system, brain tumors, and even glaucoma and ischemic eye diseases.¹ The release of excitatory amino acids, particularly glutamate and aspartate, as a response to neurological injury, connects these seemingly unrelated disorders.

After being first described and given the name excitotoxicity in 1969 by John Olney, the molecular mechanism has evolved to include at least five types of receptors, each acting in a different manner.² We know the most about the NMDA type, a voltage gated receptor that is regulated by glutamate, glycine, zinc and magnesium. It also contains a phencylidine (PCP) domain. Three of the glutamate receptors regulate calcium channels and the other two, called metabotropic receptors, act via G-proteins, at least one of which is neuroprotective. The excitotoxic process involves the delayed death of excitotoxin exposed neurons based on the accumulation of excess calcium into the cell's interior, triggering a series of destructive processes involving inflammatory pathways. This then leads to cytokine activation and free radical injury to cellular components. There is growing evidence that various nutraceuticals offer significant protection against this excitotoxic process.³ This paper will review the basic understanding of the neurodegenerative process and selected instances concerning nutraceutical neuroprotection.

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THE CENTRAL ETIOLOGY OF NEURODEGENERATION AND BRAIN INJURY

Excitotoxicity appears to be a central feature of many types of pathological processes involving the central nervous system. It is now known that pathologic injury to the nervous system from any causation triggers a release of glutamate from its storage sites.⁴ Under physiological conditions the glutamate engages the neuronal receptor, opening the calcium channel for a fraction of a second. The amount of glutamate normally allowed in the extracellular space is minute, and is carefully regulated. After allowing a small amount of calcium into the cell's interior, the glutamate is quickly bound to a carrier molecule, the glutamate transporter protein, and transported back to the astrocyte. There are a family of five glutamate transporter proteins found throughout the brain each with levels of expression and type specific locations.^{5,6}

With severe injuries to the brain the amount of glutamate in the extracellular space may reach levels 100X that normally seen.^{7,8} This can overwhelm the glutamate transporter system, resulting in prolonged activation of the glutamate receptor with a resultant excess calcium entry into the neuron. Normally, intracellular calcium is carefully regulated within the cell, either by extrusion from the cell or by trapping by the mitochondria or endoplasmic reticulum.⁹ In cases of cellular dysfunction, as seen with ischemia/hypoxia, hypoglycemia and neurodegenerative disorders, these homeostatic mechanism malfunctions lead to calcium accumulation intracellularly. Elevated levels of calcium can trigger activation of protein kinase C with subsequent expression of membrane bound phospholipase A2 triggering the release of arachidonic acid from the cell membrane. Arachidonic acid is then acted on by lipoxigenase and cyclooxygenase I and II, leading to the generation of leukotrienes and prostaglandins (PGE2, PGD2).¹⁰

These eicosanoids are responsible for a cascade of inflammatory reactions that include the generation of reactive oxygen species and inflammatory cytokines (IL-1 β ,

IL-6, TNF-alpha).¹¹⁻¹² These reactive oxygen species damage cellular proteins (protein carbonyl products), DNA and lipids (lipid peroxidation). Intracellular calcium also induces and activates nitric oxide synthase (NOS) which, when overactive, can lead to the formation of the powerful nitrogen radical peroxynitrite formed from a reaction of nitric oxide with the superoxide radical.¹³ Peroxynitrite can enter the mitochondria and damage the electron transport enzymes, especially complex I and IV, leading to impaired energy production by the mitochondria, making the neuron infinitely more susceptible to excitotoxicity. The generation of increased levels of free radicals within the cell, if intense enough, can activate the p53 tumor suppressor gene triggering apoptosis.¹⁴ Excess glutamate can also kill neurons by necrosis as well.

WHAT TRIGGERS GLUTAMATE RELEASE?

A multitude of events can trigger glutamate release, including microglial activation, inflammation, toxic metals, hypoxia, ischemia, hypoglycemia, trauma, and magnesium deficiency. It has been shown that an early reaction to ischemia, hypoxia and hypoglycemia is the overwhelming release of excitatory amino acids, glutamate and aspartate, that can persist for prolonged periods of time.¹⁵ Brain glutamate levels measured following blunt trauma to the head have shown elevated levels persisting as long as seven days.¹⁶

One of the real puzzles is, what initiates the excitotoxic cascade in neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and ALS? One event appears to be an elevation in free iron levels associated with aging and certain pathological conditions. Elevated free iron, especially in the presence of ascorbate, is known to trigger the formation of the powerful hydroxyl radical. Free radicals themselves have been shown to increase the release of glutamate from astrocytes, further elevating free radical generation. Recent studies indicate that those with Parkinson's disease have a defect in iron metabolism that favors free iron accumulation in the striatum.^{17,18} Elevated levels of free iron have also been demonstrated in the spinal motor neurons of ALS patients and the hippocampus of Alzheimer's patients.

Of increasing interest is the relationship between inflammation and the neurodegenerative disorders. For example, elevated levels of IL-1 β , IL-6 and TNF-alpha have been found in all three conditions.¹⁹⁻²¹ Several reviews have reported a reduction in the incidence of Alzheimer's disease with the prolonged use of anti-inflammatory medications.²²⁻²⁴ In one such review, a reduction of 6 to 12X was seen in users of NSAIDs.²⁵ The question that remains is; What started first, the inflammatory cascade or a pathological process that led to an autoimmune reaction?

With increasing evidence that beta-amyloid accumulation is not the primary cause of Alzheimer's dementia nor

Lewy bodies Parkinson's disease, it may be that an infectious process is acting as the trigger of this pathological series of events. A family of viruses or a species of mycoplasma are possible culprits.^{26,27} Mycoplasma have been isolated from cases of Parkinson's disease and other neurological disorders.²⁸ In the presence of immune incompetence, the attempt by the immune system to eliminate the organism would be impaired, leading to a chronic smoldering inflammatory response which includes elevated reactive oxygen and nitrogen species. A constant elevation of ROS/RNS has been shown to trigger glutamate release from activated microglia as well as from astrocytes.²⁹ Significant free radical alteration of cellular membrane proteins could initiate an autoimmune reaction as well.³⁰ Evidence supporting such an etiology remains to be proven.

FACTORS KNOWN TO INCREASE EXCITOTOXICITY

- Inflammatory eicosanoids
- Free radical accumulation
- Excess unbound iron and copper
- Magnesium deficiency
- Zinc deficiency
- 4-hydroxynonenal
- Cytokines (IL-1 β , IL-6, TNF-alpha)
- Cellular energy deficits (ischemia/hypoxia/hypoglycemia)
- Antioxidant deficiency
- Glycine excess
- Immaturity of the CNS

Table 1

EXOGENOUS GLUTAMATE AND ASPARTATE FROM PROCESSED FOODS

In 1958 Lucas and Newhouse demonstrated that MSG given to mice could produce severe destruction of the inner nerve layer of the retina.³¹ Approximately ten years later, on repeating the experiment, Dr. John Olney discovered that critical areas of the brain were also severely damaged, particularly the arcuate nucleus of the hypothalamus.³²

Subsequent experiments have confirmed this observation and that additional brain areas could be damaged including the other hypothalamic nuclei, striatum, hippocampus, amygdala, cerebellum, locus ceruleus, and circumventricular organs.³³⁻³⁶

Recently, it has been shown that MSG exposure early

in life can lead to the development of abnormal pathways in the brain, especially in the visual system and hypothalamus.³⁷⁻³⁹ This could lead to abnormal function of the neuroendocrine system and complex neural networks on a long-term basis. It should be appreciated that during the nine months of pregnancy, what the mother eats has a significantly strong potential of adversely affecting the developing fetal brain. This is especially true in light of the finding that MSG not only passes through the placenta but undergoes a two fold higher concentration in the fetal brain as compared to the maternal brain.⁴⁰

The immature nervous system has been shown to be 4X more sensitive to excitotoxicity than is the mature CNS.⁴¹ Interestingly, humans are the most susceptible animal species found, being 5X more susceptible to excitotoxicity from MSG ingestion than is the mouse, the next most sensitive species. Humans are 20X more susceptible than the rhesus monkey.

We know that the brain undergoes its most rapid growth phase during the last trimester of pregnancy and the first months of life. By age four the brain has reached 80% of its adult size and by age eight, 90%. During this rapid growth period the brain is especially sensitive to exogenously fed excitotoxins, since the protective antioxidant enzymes, the blood-brain barrier and special cystine uptakes systems are all immature.⁴²

Some have claimed the brain is protected against MSG toxicity by the blood-brain barrier. But independent studies have shown that with chronically elevated blood levels, glutamate can penetrate even the normal barrier.⁴³⁻⁴⁵ Several areas of the brain, the hypothalamus and the circumventricular organs, have no barrier and provide a back door for glutamate entry into the brain. Likewise, there are numerous conditions under which the barrier is broken down. For example, breeches in the barrier have been seen with hypertension, diabetes, trauma, brain tumors, radiation treatments, strokes (minor as well as major), Alzheimer's disease, multiple sclerosis, stress, exposure to certain drugs-legal and illegal, subarachnoid hemorrhage, and by elevated glutamate itself. A recent report found that ROS/RNS increases blood-brain barrier opening.⁴⁶

Soon after World War II, American food processors began to add substantial amounts of MSG to the food supply, primarily to enhance the taste. The amount of excitotoxins being added to processed foods is significant and can easily reach levels, especially in small children and the unborn, that equal those used in research to produce nuclear destruction.⁴⁷ Between 1945 and 1968, MSG was being added to baby foods that equaled the amount used to produce these lesions experimentally. The industry agreed to cease the practice only after a congressional hearing exposed the dangers involved. Today, baby formula frequently utilizes caseinate that contains a significantly high

enough level of glutamate to endanger a newborn's brain.⁴⁸

Briner and Freida conducted a two part study in which they fed pregnant mice MSG during their pregnancy, and then tested the offspring using both simple mazes and more complex mazes.⁴⁹ They found that the animals could perform the simple mazes without difficulty, but had great difficulty completing the more complex testing. In a second part of the study they measured neurotransmitter levels in the animals at various ages and found that during early development, and extending into early adulthood, the animals showed an 80% reduction in acetylcholine and a later fall in norepinephrine in the frontal areas of the brain.⁵⁰ Other experiments have confirmed the negative effects of post natal feeding of MSG on behavior and learning.⁵¹⁻⁵³

Neuroendocrine dysfunction has been shown by several independent researchers, involving a loss of growth hormone pulsation and reduced levels of LH, FSH, TSH and prolactin.⁵⁴⁻⁵⁷ Smaller doses of MSG induced premature puberty in the female animals.⁵⁸

One of the early observations with MSG exposure to newborns was that they became morbidly obese. This has been repeated in virtually all subsequent experiments. During a recent international neuroscience conference, a European neuroscientist asked if the high contents of MSG in the US food supply could explain the high incidence of obesity in the United States. We know that childhood obesity has increased 600% in the last ten years in this country. While not proven, in the face of the enormous amounts of glutamate and other food based excitotoxins being consumed, especially by the young and even infants, it deserves a careful look.

Experimentally, we know that the temporal lobes are one of the more sensitive cortical areas to neurotoxic injury due to elevated levels of glutamate. The distribution of neuronal damage is very similar to that seen with Alzheimer's dementia.⁵⁹ It has also been suggested that the basal nucleus of Meynert is one of the central sites of damage in Alzheimer's disease. This nucleus contains numerous glutamate-type neurons and sends cholinergic fibers diffusely throughout the brain. When cortical neurons are exposed to MSG they produce ALZ-50, 5E2 and ubiquitin, all considered to be markers of Alzheimer's disease.⁶⁰ MSG can induce neurofibrillary tangles in neurons in culture as well.⁶¹ Excitotoxic connections to Parkinson's disease, Huntington's disease and ALS have also been demonstrated.⁶²

All of this evidence points to excitotoxicity as a central process in the pathology, but does not indict ingested glutamate as the culprit. But, from what we know about the toxicity of ingested MSG and other dietary excitotoxins, they could accelerate the onset of these conditions and intensify them. Recent studies have shown that MSG ingestion can increase free radical generation in a multitude of tissues other than the brain, and in the brain may produce

prolonged ROS production even after the MSG dose has been stopped.⁶³⁻⁶⁵ With the strong connection between excitotoxicity and free radical injury one can see that ingesting MSG or other excitotoxin food additives represent a distinct danger to either those having one of the neurodegenerative diseases or at high genetic risk.

The food processing industry frequently disguises the names of the various excitotoxin additives, using such names as hydrolyzed vegetable protein, protein isolate, protein extracts, caseinate, and natural flavorings.⁶⁶ Since 1948 the food processors have doubled the amount of MSG added to foods every decade. When combined with other known neurotoxins found in our daily environment, such as fluoride, aluminum, iron overload, organophosphate pesticides and herbicides, and aspartame, we see that the brain becomes quite vulnerable to injury.

PHYTONUTRIENTS AND METABOLIC STIMULANTS: THEIR ROLE IN NEUROPROTECTION

While the use of Phytonutrients as preventatives of a host of degenerative diseases has been proposed, one area that has been all but over looked clinically is their use in neurodegenerative diseases. In 1989 the results of the DATATOP (Dephrenyl and Tocopherol Antioxidant Therapy of Parkinsonism) study was completed as part of a large multicenter, controlled trial evaluating the use of 2000iu/day of dl-alpha-tocopherol and 10mg/day of deprenyl.⁶⁷ It was concluded from this study that vitamin E had very little effect on the course of the disease.

This study was unfortunate in that they used a single antioxidant and a form of vitamin E, dl-alpha tocopherol, that has been shown to have less antioxidant power than D-alpha-tocopherol (RRR-alpha-tocopherol).⁶⁸ In a subsequent study reported in the *Annals of Neurology* in 1992, Stanley Fahn found that supplementing early stage Parkinson's patients with 3,200 iu/day of vitamin E (the form was not disclosed), and 3000mg /day of vitamin C could delay the onset of a need for levodopa by 2 to 3 years.⁶⁹ This was a pilot study and not controlled.

Basic research has shown several avenues in which phytonutrients may hold great promise in reducing the incidence of neurodegenerative diseases and possibly even treating the established disease. With the above knowledge of the neurodegenerative process, we see that any agent that blocks excitotoxicity, reduces the inflammatory cascade, supports neuron energy production, enhances the repair process and protects the CNS structures from reactive oxygen and nitrogen species, can supply this protection.

PROTECTION AGAINST REACTIVE OXYGEN AND NITROGEN SPECIES

We now know that under normal conditions the major-

Neuroprotective Effects of Phytochemicals

- Powerful free radical scavengers for many radicals
- Iron and copper chelation
- Improves membrane fluidity
- Increased neuronal glucose uptake
- Regeneration of other antioxidants
- Restoration of cellular glutathione
- Inhibition of LOX and COX enzymes
- Inhibition of phospholipase A2
- Direct glutamate receptor blockade
- Restoration of blood-brain barrier
- Improved cerebral blood flow
- Protection of vascular endothelium
- Receptor restoration

Table 2

ity of free radicals are produced within the mitochondria. With 95% of the cell's oxygen entering the mitochondria to engage in the production of energy substrates, approximately 3 to 5% is diverted to the formation of free radicals. A portion of these radicals participate in physiological reactions, but the excess can harm the cell's contents. Basically, three components of the cell are damaged by reactive oxygen and nitrogen species: the DNA, proteins, and lipids. DNA oxidation occurs earlier because of its close proximity to the generation process. Mitochondrial DNA is 10X more vulnerable than is nuclear DNA.⁷⁰ After age 70 it is 15X more vulnerable.

The result of DNA injury can be minor or involve multiple deletions, chromosomal breaks or translocations, with the resultant activation of destructive genes such as the p53 and p38 genes.⁷¹ Importantly, injury to mitochondrial proteins (such as complex I and IV) can impair the cell's ability to produce energy, leading to the amplification of excitotoxic injury.⁷² In fact, when energy supplies are low, even normal levels of glutamate can be excitotoxic.⁷³

Cellular proteins become quickly involved in the oxidative process leading to the formation of protein carbonyl products. In addition, RNS can produce nitrotyrosine products, both of which have been increased in neurodegeneration.⁷⁴ Many cellular proteins are enzymes, so that oxidative/nitration injury can lead to major disruption of the cell's function. By reducing protein oxidation, antioxi-

dants preserve DNA reparative enzymes.⁷⁵ Preserving DNA reparative systems plays an important role in resisting many of the effects of neurodegeneration, such as mitochondrial energy failure.

Finally, ROS and RNS interact with the lipid membranes of the cell initiating lipid peroxyl and hydroperoxide formation, that is, lipid peroxidation. As this reaction propagates throughout the membrane secondary products are created called lipid peroxidation products that can initiate further ROS generation or damage glutamate transporter proteins, thereby accelerating and prolonging excitotoxicity.⁷⁶ It should also be appreciated that the cell membrane contains numerous components including transport proteins, ion channels, gap junction communication channels, and various glycoproteins and glycolipids that can all be damaged by the peroxidation process. The result is accelerated degeneration of the neuron.

The antioxidants operate at different levels within the cell. Some lie in close approximation to the DNA (quercetin, epigallocatechin gallate), others function within the cytosol (magnesium, ascorbic acid, carotenoids), and some operate primarily within the lipid portion of the cell (tocopherols, tocotrienols, vitamin D, and the carotenoids).

All of these ROS/RNS processes have been shown to be accelerated in the neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and ALS.⁷⁷⁻⁷⁹ Of particular interest is the finding of elevated levels of the lipid peroxidation product 4-hydroxynonenal (4-HNE) in all three conditions. This product has been shown to not only impair mitochondrial function but also oxidizes the glutamate carrier proteins, leading to accumulation of glutamate in the extracellular space for prolonged periods of time. In addition, 4-HNE has been shown to inactivate glutathione reductase and prevent dephosphorylation of hyperphosphorylated tau by binding directly to the tau protein.⁸⁰ Hyperphosphorylation of the tubular tau protein is considered to play a central role in Alzheimer's disease. Most antioxidants have no effect on 4-HNE. Only glutathione and several of the flavonoids have shown significant scavenging ability against 4-HNE.⁸¹ When 4-HNE is injected into the mouse brainstem, glutathione levels fall in a dose dependent manner.

Flavonoid intake can be increased through a diet high in fruits and vegetables or by supplementation with fruit and vegetable extracts. Most of the flavonoids are powerful free radical scavengers, acting against a variety of ROS and RNS. For example, in order of effectiveness, the following phenolics have been shown to significantly inhibit peroxynitrite tyrosine nitration: caffeic acid, chlorogenic acid, ferulic acid, P-coumaric acid, O-coumaric acid, and M-coumaric acid.⁸²

Other flavonoids can protect neurons by inhibiting the release of astrocytic nitric oxide, a source of the powerful

radical peroxynitrite. Using in vitro techniques, Soliman and colleagues found a long list of flavonoids that could accomplish this goal. These include: quercetin, (-) epigallocatechin gallate, morin, curcumin, apigenin, sesamol, chlorogenic acid, fisetin, (+) taxifolin, (+) catechin, ellagic acid and caffeic acid in order of potency.⁸³ Products inhibiting NO production at concentrations less than 300 ppm included: silymarin, grapefruit, and green tea. Compared to the known peroxynitrite scavenger ebselen, flavonoids were found to be 10X more effective in scavenging this powerful radical.

It is hypothesized that in the case of Parkinson's disease dopamine breakdown products are produced by MAO-B generated peroxide radicals in the vicinity of the substantia nigra. The MAO-B inhibitor, D-deprenyl, acts in part by inhibiting this enzyme. A number of flavonoids have also been found to significantly inhibit MAO-B, including: Chlorogenic acid, (+)catechin, taxifolin, (-) epigallocatechin gallate, fisetin, coenzyme Q10, curcumin, sesamol, morin, sesame oil, silymarin, green tea, caffeic acid, and rutin hydrate.⁸⁴

Glutathione is a sulfhydryl containing molecule that is present in all cells including neurons, as a major scavenger of ROS and RNS. Several events are known to reduce glutathione levels in cells, including a reduction of other antioxidants during oxidative stress, low magnesium levels, and exposure of neurons to glutamate. The latter can markedly lower cellular glutathione levels.⁸⁵ Cellular protection can be increased by enhancing glutathione levels. Several methods have been found to accomplish this including increasing ascorbate levels, alpha lipoic acid supplementation and increased intake of precursors such as cystine and N-acetyl-L-cysteine. Glutathione supplements are a poor way to increase cellular levels since glutathione cannot enter the neuron. As a result, the molecule is broken down into L-cysteine which is a weak excitotoxin. While cystine is a safe form to use for enhancement, L-cysteine is not. There is evidence that a cell's ability to survive an oxidative insult is directly proportional to its ability to manufacture glutathione on demand and not its absolute levels.

The brain has one of the highest levels of ascorbate in the body and all animal species examined have been found to have high brain ascorbate levels in the cortex and white matter.^{86,87} In fact, the brain concentrates ascorbate so that levels are 40X higher in the brain than the plasma. Neurons and glia have mechanisms that actively transport ascorbate into the cells in even higher concentrations. Ascorbate in the CNS acts not only as an antioxidant but also plays a role in neurotransmission and participates in a glutamate heteroexchange system. In a recent study, Rivere and co-workers found that vitamin C plasma levels were significantly lower in Alzheimer's patients despite adequate dietary intake and that deficient vitamin C plasma levels correlated with the degree of cognitive impairment.⁸⁸

Vitamin E levels remained stable.

Brain levels of vitamin E are located in the lipid fractions and are very stable even after prolonged dietary deficiencies. A high intake of polyunsaturated oils can lead to pathological lesions of the brain secondary to tocopherol deficiencies as can chronic malabsorption syndromes. Many years ago it was shown that supplementation of the diet with flavonoids could protect against loss of vitamin E and C. Scurvey is very difficult to induce in the face of high bioflavonoid intakes. This is important in light of the evidence that certain vitamin deficiencies are associated with reduced cognitive function. For example, Goodwin and co-workers in examining the status of vitamin C, B-12, riboflavin, and folic acid in 260 healthy subjects over the age of sixty found a correlation between poorer results on a sensitive measure of concept learning and low normal values for these vitamins.⁸⁹

In a subsequent study in which cognitive performance and EEG power were measured during cognitive task, it was found that subclinical low levels of certain vitamins were associated with poor cognitive function.⁹⁰ One third of the group had marginally low thiamine levels with associated less alpha activity in the frontal, temporal and parietal lobes, a conventional sign of normal EEG described brain function. A greater number of significant relationships were seen between plasma carotene levels and cognitive performance and EEG power. Surprisingly, no correlation was seen between iron status in these elderly subjects and cognitive function, as is seen in younger subjects. As carotene had the strongest correlation, this might indicate that antioxidant protection of the brain plays a vital role in cognitive preservation with aging. The etiology and pathophysiology of age associated memory impairment has not been elucidated.

In a recent study using four neuropsychological tests and estimations of frontal lobe volume using the MRI, it was found that those with age associated memory impairment (AAMI) had no evidence of decreased frontal lobe brain volume as compared to aged and educationally matched controls, but did significantly poorer on 3 of 4 frontal lobe function test (Indicating a loss of memory and executive function). This would indicate physiological neuron dysfunction rather than neuron loss as seen in dementia. Only a small portion of those having AAMI will eventually develop dementia.⁹¹ Such physiological dysfunction has the potential of being reversed. There is evidence that excessive glutamate either intrinsic or extrinsic can have deleterious physiologic effects without cell death.

Oxidative damage to DNA is seen in all of the neurodegenerative disorders, especially to mitochondrial DNA which, as pointed out, is 10X more sensitive to oxidation than is nuclear DNA.⁹² DNA protection against oxidative injury has been demonstrated with several of the vitamins, such as ascorbic acid, carotenoids and vitamin D. Of even

greater interest are some of the flavonoids, such as quercetin,(-) epigallocatechin gallate and Egb 761(Ginkgo biloba extract).^{93,94} Most of the antioxidant flavonoids indirectly protect DNA by reducing peroxytrite, superoxide and hydroxyl radicals. It has been shown recently that quercetin demonstrates even greater DNA protection in the presence of vitamin C and that it accumulates in the perinuclear area.⁹⁵

A recent study found that all flavonoids tested and vitamin C produced a dose dependent reduction in oxidative damage to human DNA.⁹⁶ From most effective to least effective were luteolin (91% protection), myricetin (90% protection), quercetin (78% protection) , kaempferol (68% protection), apigenin (41% protection), rutin (18% protection) and vitamin C (22% protection). The protective effects of the flavonoids and vitamin C were additive.

MITOCHONDRIAL ENERGY STIMULATION

One of the earliest changes in Alzheimer's disease and Parkinson's disease is a decline in neuronal energy production in the regions involved in the pathology- frontal, inferior parietal and temporal. This can be seen several years before the clinical onset utilizing the PET scan.⁹⁷ Distinct enzyme defects have been mapped out in both disorders, complex I deficiency in Parkinson's disease and complex I and IV with Alzheimer's disease.⁹⁸⁻¹⁰⁰ Both of these enzyme defects occur in the mitochondria and are thought to be damaged by ROS and RNS, especially the peroxytrite radical.

The designer drug MPTP induces explosive Parkinsonism by inhibiting complex I enzymes in the substantia nigra.¹⁰¹ Measurement of complex I in Parkinson's disease indicates a reduction of about 43% of its function.¹⁰² With reduced energy production by the mitochondria we see increased free radical generation and dysfunction of cellular calcium homeostasis. There is evidence that coenzyme Q10 supplementation can by-pass complex I defects as can succinate and β -hydroxybutyrate.¹⁰³ This would increase complex II activity restoring mitochondrial energy production.

More recently, Ruiz and co-workers, using cortical neuronal cultures have shown that pyruvate and malate protect hippocampal and cortical neurons against delayed cell death occurring 24 hours after glutamate exposure.¹⁰⁴ The normally high intracellular calcium levels seen after glutamate exposure were significantly reduced by this treatment.

Treatments of various mitochondrial disorders, MELAS, MERRF and Leber's hereditary optic atrophy with metabolic stimulants have shown varying success.¹⁰⁵ These include thiamine, riboflavin, phylloquinone, menadione, tocopherols, folates, ascorbic acid, succinic acid, acetyl-L-carnitine and lipoic acid. An additional effect of metabolic stimulation is the enhancement of cellular ener-

gy-dependent processes, such as transport, cell division, and a multitude of other cell functions. This will allow the injured cell to repair itself.

Magnesium also plays a vital role in neuronal protection. Studies have shown that low magnesium levels can double the production of free radicals and magnify their toxicity.¹⁰⁶ Cells deficient in magnesium exposed to free radicals show a rapid depletion of glutathione levels. It should also be appreciated that one of the more common glutamate receptor types, the NMDA receptor, is blocked by magnesium when the cell is at rest. Magnesium also plays a vital role in hundreds of enzymatic reactions many of which are energy producing.

Methylcobalamine has been shown to directly block the glutamate receptor as has pycnogenol, both in a dose dependent manner.¹⁰⁷ In addition, pycnogenol contains powerful antioxidants and both methylcobalamine and folic acid play vital roles in DNA repair. Lipoic acid and dihydrolipoic acid (DHLA) have been shown to be protective against NMDA or malonic acid lesions of the striatum.¹⁰⁸ Flint Beal and co-workers found that a combination of coenzyme Q10 plus niacinamide could block excitotoxic lesions produced by injecting mitochondrial toxins into the striatum.¹⁰⁹

An additional benefit of certain flavonoids is their anti-inflammatory properties through their ability to inhibit cyclooxygenases and lipoxygenases.¹¹⁰ This is especially important in the case of Alzheimer's disease, since a connection has been shown between inhibition of prostaglandin synthesis and disease incidence. We know that glutamate enhances both the release of arachidonic acid from neuron membranes and enhances COX II activity.^{111,112} The advantage of flavonoid inhibition of eicosanoids is that it is not associated with gastric ulceration, hepatic or renal injury.

CONCLUSIONS

The full scope of phytopreventative nutraceuticals is beyond this paper but this limited review demonstrates the need to apply what we know concerning their protective effects and to explore other functional food components for neuroprotectant properties as well. Early attempts at effecting the course of neurodegenerative disorders utilizing antioxidants have met with only modest success. But these attempts were very crude and used single antioxidants, frequently ones known to possess reduced activity.

We know that the ROS/RNS react within different compartments of the cells and some are inactivated only by a limited number of antioxidant substances. Recent studies have shown that the flavonoids possess a wide range of antioxidant activity against a variety of reactive molecules, including peroxynitrite and lipid peroxidation products.

A frequent mistake is to assume that a failure to

demonstrate lipid peroxidation indicates an absence of oxidative stress, when it is known that free radical damage can occur individually within any cell compartment and may involve primarily protein and/or DNA oxidation in isolation.

The versatility of nutraceuticals in attacking the many molecular levels involved in the neurodegenerative process holds great promise in solving this dilemma. Treatment of these deadly disorders will require agents that can reduce inflammation, block glutamate receptors, protect glutamate transporters, improve membrane fluidity, scavenge free radicals, reduce lipid peroxidation, chelate iron and copper, and increase cellular energy production. Nutraceuticals of various types have demonstrated all of these properties.

Of particular interest is the ability of nutritional supplements to enhance cellular energy production. Energy failure in the brain is a common finding with aging, ischemia/hypoxia, hypoglycemia, vitamins/mineral deficiencies, and prolonged oxidative stress. The ability of certain metabolic supplements to by-pass enzymatic defects found within the mitochondria is of particular interest in Parkinson's disease and Alzheimer's disease. There is evidence that some of the nutraceuticals can improve membrane fluidity, stimulate receptor populations and re-establish cell-cell communication. Much remains to be explored.

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The Pharmacology of Saw Palmetto in Treatment of BPH

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There is substantial evidence that preparations of the berries of saw palmetto, *Sereno repens* (Bartram) Small are effective in alleviating the symptoms of benign prostatic hyperplasia (BPH), a nonmalignant enlargement of the prostate. However, the pharmacological mechanism(s) of this action is uncertain and investigations are complicated by the fact that the cellular mechanisms for the pathogenesis of BPH are also unclear.

Factors implicated in the pathogenesis of BPH are the presence of 5-alpha-dihydrotestosterone (DHT), an active metabolite of testosterone, and aging. BPH is also associated with an increase in the activity of 5-alpha reductase, the enzyme responsible for the conversion of testosterone to DHT. Levels of DHT are not increased but the density of androgen receptors may be. DHT has a greater affinity for androgen receptors than testosterone and is thought to be the key androgen which modulates prostatic growth. Hormone levels in men alter with aging. Testosterone levels decrease while estrogen levels remain constant. This change in the hormonal ratio of testosterone to estrogen has been implicated in BPH as estrogens are found to produce hyperplasia in animal experiments. Also implicated in BPH is a non-bacterial inflammation of the prostate (non-bacterial prostatitis). Although BPH is associated with prostate enlargement, studies have shown that the size of the gland is not necessarily indicative of obstruction of the urethra and the symptoms associated with BPH.¹

Predominant pharmaceutical treatments of BPH include alpha-receptor blocking agents (e.g. prazosin, tera-

zosin) and 5-alpha reductase inhibitors (e.g. finasteride). Alpha-receptor blocking agents are thought to be effective due to their ability to relax smooth muscle in the bladder neck and within the prostate. 5-alpha reductase inhibitors prevent the transformation of testosterone to 5-alpha-dihydrotestosterone (DHT), thus reducing levels of DHT.

Clinical studies have demonstrated that saw palmetto preparations cause significant improvement in BPH symptoms as scored by the International Prostate Symptom Score (IPSS). The IPSS is derived from answers to a questionnaire on symptoms of urgency, daytime and nighttime urinary frequency, hesitancy, intermittency, sensation of incomplete voiding and force of urine stream. Decreases in IPSS scores have been reported following administration of 320 mg extract per day for 1 to 2 months with further improvement reported after 3 months or longer.²⁻⁴

Administration of 160 mg extract (Prostaserene®) twice daily was compared to administration of 320 mg once a day in multicenter, single-blind, parallel randomized study. This study, which entered 84 patients with a maximal urinary flow of greater than 5 ml/s but less than 15 ml/s, reported the two treatments to be therapeutic equivalent. Both treatment regimes produced an increase in maximal and mean urinary flow rates measured on day 30 ($p < 0.0001$ and $p < 0.01$ respectively). Residual urine decreased with an initial greater drop at day 30 compared to day 180 and 360. Prostatic volume decreased significantly following 90 days of treatment ($p < 0.0001$).⁴

Long-term treatment with saw palmetto was studied in a three year open-label prospective multicenter study with 435 male patients (diagnosis of stage I or II) administered 160 mg bid of extract IDS 89 (Strogen®). Both physicians and patients rated the efficacy of treatment as good or very good in over 80% of the 315 cases who completed the trial. Nocturia improved or normalized in 73% of patients over three years. Daytime frequency improved and the feeling of incomplete emptying for 54% and 75% of patients

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respectively. Residual volume fell from an average of 64 mls to 38 mls during the first three months of treatment. Peak flow rose during the first 12 months and then stabilized. The average increase in peak flow rate was 6.1 ml/s at the end after 36 months. The deterioration rate was 8.8% after 12 months, 13.0% after 24 months and 14.7% after 36 months. Side effects were minimal with 30% of the 46 adverse events reported in 34 patients being due to gastric disturbances.⁵

Improvement in IPSS scores was reported even in the absence of a measurable change in peak urinary flow or postvoid residual urine volume. A non-randomized, open-label study with 50 men reported that 46% of patients reported at least a 50% improvement in their IPSS score following treatment with 160 mg bid extract (Nutraceutical Corp.). However, there was no significant improvement in mean peak urinary flow rate, postvoid residual urine volume, or bladder pressure.²

Importantly, the trials reported above did not find that treatment with saw palmetto caused any significant change in serum levels of prostate specific antigen (PSA).^{2,5}

Comparison of the efficacy of saw palmetto extract to that of finasteride was made in a 6-month, double-blind randomized equivalency study. The trial enrolled 1,098 men over the age of 50 with moderate BPH. Patient received either 160 mg extract (Permixon®) twice daily or a single daily dose of 5 mg finasteride (Proscar®) for 26 weeks. Both treatment were determined to decrease the symptoms of BPH in about two-thirds of the patients. In contrast, finasteride was more effective in decreasing prostatic volume, an 18% decrease compared to 6% decrease. Further, finasteride treatment decreased serum PSA levels by 41%, while Permixon® treatment did not alter PSA levels. Complaints of decreases in libido and impotence were more common in the finasteride group than those treated with the saw palmetto extract.³

Suggested pharmacological actions of saw palmetto preparations include anti-androgenic activity (inhibition of the conversion of testosterone to DHT by 5-alpha reductase and inhibition of binding of DHT to androgen receptors), anti-inflammatory activity, anti-proliferative activity and relaxation of smooth muscle.

Inhibition of 5-alpha reductase activity by saw palmetto preparations has been demonstrated in numerous cell culture systems including human foreskin fibroblasts and human prostate cells.⁶ Inhibition of enzyme activity in a homogenate of human BPH tissue was reported for an extract identified as IDS 89 (Strogen®) with an IC₅₀ of 2.2 mg/ml.⁷ Inhibition of isolated isoenzymes type 1 and 2 of 5-alpha reductase was demonstrated using an n-hexane extract, LSESr (Permixon®) with IC₅₀s of 4 µg/ml and 7 µg/ml respectively.⁸ The inhibitory action of IDS 89 and LSESr was found to be noncompetitive in nature and the

authors of both studies suggested that the extracts caused a non-specific modulatory action on the membrane environment of the enzymes.^{7,8} In comparison, finasteride and tursoide selectively and competitively inhibited the type 2 isoform of 5-alpha reductase with IC₅₀'s of 11 and 18 nM respectively.⁸

Inhibition of binding of dihydrotestosterone to androgen receptors has been reported in cell cultures of human foreskin fibroblasts.⁹ Studies with rat prostate receptors demonstrated competitive inhibition of binding to cytosolic receptors with an IC₅₀ of approximately 370 µg/ml of LSESr.¹⁰ Another study reported no inhibition of DHT binding to the rat prostatic androgen receptor with several extracts of saw palmetto, including LSESr, with concentrations of up to 100 µg/ml.¹¹

Inhibition of the production of inflammatory mediators has been reported. In particular, inhibition of the production of 5-lipoxygenase metabolites (5-HETE and LTB₄) in calcium ionophore (A23187) stimulated human neutrophils at a concentration of 5µg LSESr /ml.¹² Reduction of experimental edema in rodent models has also been reported.¹³

Inhibition of prostate enlargement in castrated rats treated with estradiol and testosterone was demonstrated following the addition of a saw palmetto extract (LSESr).¹⁴ That same preparation also inhibited basic fibroblast growth factor (b-FGF) induced proliferation of human prostate cell proliferation at concentrations of 10 and 30 µg/ml. Examination of constituents of the extract revealed that lupenone, hexacosanol and an unsaponified fraction demonstrated activity.¹⁵

Relaxation of smooth muscle tissue has been demonstrated by an ethanolic extract (Madaus) in contractions produced by potassium chloride on rat uterus and by nor-epinephrine on rat aorta tissue with EC₅₀'s of 350 and 530 µg/ml, respectively.¹⁶ Vanadate-induced contraction of rat uterus was inhibited with an EC₅₀ of 11 µg/ml.¹⁷

In evaluating the above studies it is important to correlate the concentration of extract and constituents used in the studies with levels expected in plasma and tissues following administration of a standard dose. A bioavailability study indicated peak plasma levels of 2.6 µg/ml following administration of 320 mg extract.¹³ This study indicates that studies citing ED₅₀'s two orders of magnitude above this are unlikely to be clinically relevant.

A few clinical studies have investigated pharmacological activity directly. A study with 20 men investigated whether 320 mg/day of a liposterolic extract would produce changes in systemic hormone levels following 1 month of treatment. No change in plasma levels of testosterone, follicle stimulating hormone or luteinizing hormone was found.¹⁸ Two additional trials, each with 32 healthy men, found no change in serum DHT levels following adminis-

tration of 320 mg Permixon® for 1 week, whereas finasteride at 5 mg/day increased serum testosterone and decreased serum DHT by as much as 65%.^{11,19} In contrast, a three month trial with 6 BPH patients given 320 mg extract per day, reported a decrease in both levels of DHT and epidermal growth factor in prostate tissue when compared with 9 patients given placebo. These decreases were also observed in 9 patients administered finasteride.²⁰ Another three month trial studying the activities of androgen metabolizing enzymes reported slight alterations in 5-alpha reductase (DHT-forming) and 3-alpha-hydroxysteroid oxidoreductase (DHT-removing) in subjects given 1.9 g /day of IDS 89. In evaluation of the results, the authors felt that the correspondence of these slight changes to clinical effectiveness was uncertain.²¹ Evidence for an inhibitory effect on androgen and estrogen receptors in prostate tissue comes from a 3 month double-blind placebo controlled trial with the treated group receiving 480 mg extract/day.²²

A few studies have explored the question of which constituents in saw palmetto preparations are responsible for the alleviation of BPH symptoms. The berries of saw palmetto contain fatty acids, fatty alcohols and sterols. Commercial preparations are commonly liposterolic extracts, made with hexane or liquid CO₂, which contain 85 to 95% combined fatty acids and sterols.

Fractionation experiments for the 5-alpha reductase inhibiting activity of extract IDS 89 found the activity to be due to saponifiable components, namely fatty acids. Non-saponifiable components, including phytosterols were not active. Evaluation of the activity of individual fatty acids, lauric acid, oleic acid, myristic acid and palmitic acid, found the greatest activity with lauric acid and myristic acid.⁷

Evidence of a role for sterols comes from clinical trials using a mixture of sterols, composed mainly of beta-sitosterol. A multi-center, placebo-controlled, double-blind 6 month study with 200 patients reported increases in peak flow and decreases in residual urinary volume with no reduction in prostatic volume following administration of 60 mg/day beta-sitosterol mixture (Harzol®). Harzol® capsules contain beta-sitosterol 10 mg (including standardized 0.1 mg of beta sitosterol-beta D-glucoside) and smaller amounts of campesterol, stigmasterol, among others.²³ A second multicenter, placebo-controlled, double-blind, 6 month study with 177 patients with BPH reported increases in flow following administration of 130 mg beta-sitosterol (Azuprostat®).²⁴

Although the beta-sitosterol content in saw palmetto preparations may play a role in clinical efficacy, the content is much less than that administered in the trials cited above. For example, a CO₂ extract produced by Indena was reported to contain 0.32% sterols.²⁵ The usual dose of 320 mg extract per day would in this case deliver 1 mg/day of

sterols. This is significantly less than the 60 mg or 130 mg administered with Harzol® and Azuprostat® respectively.

In summary, saw palmetto preparations have demonstrated efficacy in clinical trials and several modes of action have been proposed. Suggested pharmacological actions include anti-androgenic activity, anti-inflammatory activity, anti-proliferative activity and smooth muscle relaxation. Constituent fatty acids, fatty alcohols and sterols have been implicated in this activity. It is likely that the effects of saw palmetto are numerous and due to multiple constituents just as the causes of BPH are enigmatic and due to the interplay of a variety of factors.

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Standardization of Herbal Medicines: The Pandora's Box of Quality Control

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INTRODUCTION

The nutraceutical industry is booming. Like any rapid growth industry, a slew of problems present themselves at a rate that often outstrips solutions. One such issue is the standardization of herbal medicinals. Historically this was seldom an issue as one obtained herbal medicines from an herbalist who was qualified by experience or training, and who had a keen eye for the plant and its nuances. Herbal medicine, however, is no longer a cottage industry. Nowadays, we usually get our medicinals in capsules, elixirs, and other formulations far removed from the original plant source and the personal advice of a specialist. While numerous schools train and educate herbalists, these healthcare professionals also rely on products from manufacturers. Old methods of evaluation and quality control are now difficult and sometimes obsolete. How can we know that any particular product is of a satisfactory quality? Can we distinguish between manufacturers and their products? Is standardization, as currently practiced, the answer?

Standardization is like the old adage, be careful what you ask for because you just may get it! When the public asks for standardization, they are not really asking for a specific amount of a particular chemical. What they are asking for is quality control and information with which they can distinguish one product from another. Presently, new consumers are buying herbal medicines; many are unfamiliar with the old approaches of herbal medicine quality control. The general public currently comes from a background experience with pharmaceuticals. They are comfortable in the world of doses in mg of specific chemicals, an exactness that is reassuring.

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Herbal medicines do not lend themselves to milligram-specific exactness. Like the plants they are derived from, they are a collage of chemical components. Plants' evolved synergistic make-up may be the explanatory variable for their remarkable safety record—providing balance and freedom from side effects as they deliver their healing components.

So, in offering standardized products, is the industry truly meeting the public's needs? Is it offering better products? For some products yes, others no. What we are offering now is an opportunity to confuse a public that craves reliable information. This is a disservice.

There is a strong movement in the nutraceutical industry, and justifiably so, for production to be performed according to "good manufacturing practices" (GMP). This assures the public that product processing has their safety in mind. The death of individuals from some Chinese herbal medicines due to heavy metal contamination, unrelated to biological properties of the herb itself, typifies this concern. The GMP form of self-policing will improve the perception of the industry by the public and health care providers. Does standardization for specific chemical constituents provide similar assurance?

THE ASSUMPTIONS BEHIND STANDARDIZATION *Mechanisms of Action as a Prelude to Active Components*

Several assumptions made when we standardize a product are outlined in Table 1. These assumptions vary from purely academic questions related to the biomedical effects of the herb to the practices of the manufacturer. In comparison with pharmaceuticals there are many unanswered questions with herbal medicines, not the least of which are (1) how do they work? And (2) what chemical(s) or chemical classes are responsible for their action? The order of these questions is important. It is not possible to ascertain the active compounds of an herbal preparation when we do not know how that herbal medicine works. In other words, without a specific biological readout, one cannot ascertain what chemical constituents of a plant product are mediating these actions.

TABLE 1**Standardization of Herbal Medicines: Assumptions**

1. Mechanism of action of herbal medicines are known
2. Active compounds are known
3. Patented compounds offer meaningful insight into activity
4. Manufacturers use comparable assays to quantify active compounds
5. High-quality, pure standards are available
6. When supplementing an herbal product with an "active compound," the quality of the base product (herb) has not been compromised

When describing a mechanism of action it is insufficient to state it "stimulates the immune system" or other such non-descriptive observations, terms the FDA allows as descriptors of function. Specific actions like activation/inhibition of a receptor, enzyme, or gene are needed. For the majority of herbal medicines, we just do not know this information as there has been little research addressing these questions. The explanation for this is partly economic and partly historical: pharmacognosy and not pharmacology has been the pervading scientific approach. Pharmacognosy deals more with the compounds in plants as it relates to ethnobotany; pharmacology treats the extracts more like drugs, with specific consequences and effects. **Thus, when the mechanism of action of an herbal medicine is unknown, how can we state what chemical components were responsible for this effect? Until we provide a precise means of quantifying "activity", it is not possible to define the active compounds, and consequently, what are we standardizing to?**

This problem is further complicated by the reiteration of poor science. This problem plagues the nutraceutical field. Isolated observations or interpretations cited often enough take on the aura of validity. Unfortunately, we have had not had the same resources as other fields of biomedical research to re-evaluate these studies and place them in perspective. Note that the derivation of the word research is "to look again". We need to reassess and confirm seminal observations, a fundamental core process of all scientific advancement.

PATENTS VS. SCIENTIFIC VALIDATION

Related to this issue is the assumption that when a patent has been issued for a chemical constituent of an herbal medicine, then clearly it must be an important and active component. The concept here is when the government has given a stamp of approval, then it must be correct. This conclusion is a misinterpretation and reflects a naïve assessment of the patent process. To receive a patent one has only to prove novelty and be the first to show something. It does not have the same scientific scrutiny for "correctness". For example, when a new

chemical compound has been isolated from an herbal medicine and when one can show **any** form of biological activity, then one can receive a patent related to that chemical. The industry assumes next that when a new chemical has biological activity then that chemical is a component of that herb. Often this involves a close approximation to the biomedical effects of the herb by the patented compound. There are many cases where this has been correct and successful, for example, digitalis, quinine, cocaine, curare, and yohimbine. There are numerous examples where the approximation is far from complete. An excellent example is cat's claw (Uña de gato), the anti-inflammatory herbal medicine from the Amazon.

A number of patents involving oxindole alkaloids found on the bark of cat's claw¹⁻³ were issued to Austrian journalist Klaus Keplinger. During the required biological assay, Keplinger and colleagues noted that these alkaloids promoted phagocytosis--the ingestion of debris and microorganisms by white blood cells. Consequently, cat's claw was identified and touted as an immune stimulant. That step involved the assumption that these novel chemicals mimicked the activity of the ethnomedicine, a correlation that remains to be validated. Nevertheless, most literature describes cat's claw as an immune stimulant. What is difficult to appreciate with this claim is that indigenous peoples of the Amazon take cat's claw for conditions in which the immune system and leukocytes have excessive activity, e.g., arthritis, gastrointestinal inflammation, asthma. All current therapies used for these conditions focus on restoring balance to the immune system or negating the effects of specific mediators of inflammation in addition to symptomatic relief. In severe cases, powerful drugs like glucocorticoids (immune suppressants such as methotrexate, cyclosporin, azothioprine), are used to control an immune system that is attacking the host. To postulate that these conditions can be treated by a chemical that stimulates the immune system and phagocytosis is counterintuitive, if not dangerously inappropriate. In addition, the traditional water extraction method for cat's claw releases low amounts of oxindole alkaloids (as opposed to alcohol extraction) and the leaves of cat's claw are rich in alkaloids but are not used by indigenous cultures. One is left with the logical conclusion that oxindole alkaloids are not the active components of cat's claw as they do not share biological actions and biomedical applications. Nevertheless, many cat's claw products in the U.S. are standardized for oxindole alkaloids, and a high oxindole alkaloid content is regarded as being synonymous with high potency. Knowing the history, the science, and the process, one can easily discern the fallacy of this approach.

This begs the question, how does cat's claw work? We have recently discovered an action of cat's claw that supports and explains its use in inflammatory disorders.⁴ In addition to being an antioxidant, cat's claw prevents the activation of a protein that acts like a switch on the genome.

This switch, a transcription factor called NF- κ B, regulates the expression of over 28 different genes involved in inflammation. Genes that code for cytokines, adhesion molecules, chemokines, and enzymes are activated by NF- κ B. In chronic inflammation, autoimmunity, or excessive immunity, this NF- κ B switch inadvertently remains on, and the genes that it regulates are copied on a continual basis. We demonstrated that cat's claw turns this protein switch off (literally removes it from its docking site on DNA), returning these inflammatory genes to their normal quiescent, dormant state--a powerful and effective means of controlling an inflammatory response and an inappropriately activated immune system. With this information, we can now address the potential active compounds in cat's claw.

There are other circumstances where active ingredients are touted for purely marketing purposes. Again, the shroud of poor science confuses and bewilders the public and healthcare personnel. Maca, a tuber, has been used for 5800 years to restore fertility in indigenous cultures of the Andes. Recent Western world interest in Maca has led some companies to promote their Maca as being standardized for "macaenes" or "macamides", chemicals touted as the active components of maca. Disturbingly, these mystery compounds are of unknown activity and structure. Again, as the mechanisms underlying maca's pro-endocrine effects in males and females are unknown, it is not possible to assign to any compound the mantle of "active component". Some products are standardized to "marker compounds", compounds known to not be active agents, yet are present in the whole herb. The public is not given the opportunity to distinguish between standardization for marker vs. active compounds, and it opens the door to questionable marketing strategies. **Thus we are left with the realization that through our desire to provide to the public a quality product via standardization, we have established a means to perpetuate confusion and a marketing tool that can be abused.**

METHODS OF QUANTIFICATION

Other assumptions made with standardization are that the tests used to perform these assays are uniform and equivalent. Is one company's methodology equivalent to another company's? In the pharmaceutical industry this is less of an issue. In the nutraceutical field, these standardization assays may vary from crude colorimetric and simple UV absorbance assays to the more sophisticated HPLC, CE or GC-MS or LC-MS techniques. The labeling of "standardized to" reveals none of this information. Some assays are expensive and one must question advocating their application (i.e., solve the problem by applying more technology) when we are unsure as to what chemicals we should be assaying to begin with.

QUALITY OF STANDARDS

The term standardization assay implies that levels in a sample are compared to pure chemicals--the standards. However, many of these standards are incredibly complicated chemicals, ones difficult to synthesize. Hence their only source is biological. In other words, they are derived from an extract of the plant that one is comparing the unknown. While there are techniques to assist in concentrating and purifying these extracts, it remains a source of variability and cost. One manufacturer may not use the same standards as another, leading to quite different values. Again, be careful what you ask for.

INTEGRITY OF THE BASE PRODUCT

One simplifying approach is the fingerprinting technique, which uses reasonably sophisticated techniques to map out the spectrum of compounds in a plant extract. While this has merit, the fingerprint may vary greatly with different extraction procedures for the same plant base. It does however, approach the problem of manufacturers using a poor quality medicinal plant base and then adding at the end of the process a certain amount of "proposed active constituent" and then marketing this product as having superior quality or potency. There are opportunities here for disrupting the fabric of herbalism by removing compounds that may balance side-effects, provide synergistic effects, or even in the case of cat's claw, add compounds that are counterproductive.

For the most part, the public and retailers in the nutraceutical industry are oblivious to many of these issues. They want a reliable and predictable product, appropriately so. Standardization is a possible means of supplying that assurance. The question is: how do we go about it? Until these issues are addressed in a meaningful manner, standardization is more akin to opening Pandora's box, releasing more complications and problems than solutions.

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Standardizing: Does it Ensure Quality of Herbal Products?

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The materials used in botanical supplements have evolved over time from predominately raw herb/root powders to concentrates and now to standardized extracts. This progression has resulted from pressures to increase product quality and effectiveness. The question isn't whether more quality is needed, but how this quality can be assured. The components of natural products vary based on where they are grown, when they are harvested, and how they are processed.¹ Chemical standardization and clinical trials are the most common methods used to assess performances, but given the natural variance of herbal products, are these the best methods to ensure quality and consistency?

In the last decade, given the number of new entrants to the market, the marketing of supplements has become disproportionate to the amount of scientific information available about these botanicals. With the onset of increased competition, manufacturers continue to investigate how to provide higher quality, more consistent products. Unfortunately, this is a difficult undertaking considering the nature of these products. Herbal supplements may vary in phytochemical content, making it difficult even with chemical standardization, to ensure that these products can perform in every batch produced.

Chemical standardization refers to a specified level of one or two classes of chemical constituents.¹ It serves as a defined quality measure that manufacturers can use to make sure the product is what it says it is. Marker compounds used for standardization often represent a class of structurally related phytochemicals (flavonoids, ginsenosides, terpenoids, etc.) of which the crude botanical, and resulting extracts, will vary in content. Minor alterations of chemical structure can pro-

foundly impact biological effectiveness. Chemical standardization may help to ensure product content, but without corroborating biological/functional activity, the product cannot be guaranteed to work from batch to batch and bottle to bottle.²

For example, St. John's Wort extracts have been standardized to 0.3% hypericin because initial investigations found that a crude preparation of hypericin had activity against a neuronal enzyme implicated for depression. However, more recent research suggests that the active components may include a compound named hyperforin.³ As a result, extract producers have begun to adapt by producing St. John's Wort preparations containing hyperforin and hypericin. What if further study reveals other active components or that hyperforin is not the active ingredient? How will quality and consistency of St. John's Wort products be defined? Does simply ensuring content of a small percentage of the phytochemical components guarantee the compound will produce activity associated with its benefit? How is the consumer supposed to know what products work?

Well-conducted clinical trials are the gold standard in proving whether or not something truly works. Unfortunately, clinical trials test a product at one point in time and typically only test a single lot of manufactured product. This is reasonable when testing pharmaceuticals, since drugs are purified actives that work on a specific target site in the body. However, herbal products are compromised by the fact that they tend to show variability of content and activity.⁴ It is likely that herbal products will show differences in the composition and activity of each batch of product produced. The standardized components could be the same, but what if other undefined components changed in quantity? Will the combined effects be different and will that affect the ability of the botanical (product) to produce its claimed benefit?

Substantiation of product quality based upon biological activity related to its benefit claim, in addition to chemical standardization, represents a valuable quality assurance technique for complex products like herbs. Basically, functional bioassay-based testing ensures that the product 'does what it says it does' on a consistent basis.⁵ Since herbal products show variance of content based on numerous factors, the one

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consistent measure is activity. Not any activity, but specifically, biological activity associated with the product's structure/function claim.

Functional testing uses bioassays, in vitro (test tube) tests that model the biological activity of the product. They help to determine whether or not a product can produce biological activity consistently, batch to batch and bottle to bottle, associated with its claimed benefit. The design and implementation of such in vitro experimental approaches are of central importance to contemporary research and scientific discovery in pharmacology and molecular and cellular biology.

Consistent functional activity ensures not only product quality, but also product consistency. It encourages the movement forward to conduct clinical studies, in order to provide that "gold standard" of effectiveness. Because it is not possible to perform clinical trials with each lot of product, functional testing can be used to ensure consistent/equivalent biological activity. Given the inherent natural variability of

herbal/natural products, this may represent the best way to ensure product quality and consistency.

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Stephen E. Straus, MD, Appointed Director, National Center for Complementary and Alternative Medicine at NIH

HHS Secretary Donna E. Shalala recently announced the appointment of Stephen E. Straus, M.D., as the director for the National Center for Complementary and Alternative Medicine (NCCAM) at the National Institutes of Health (NIH). A nationally and internationally recognized expert in clinical research and clinical trials, Dr. Straus has served since 1991 as the Chief for the Laboratory of Clinical Investigation at NIH's National Institute of Allergy and Infectious Diseases (NIAID).

"Dr. Straus brings exceptional expertise and leadership to this position and will continue to ensure high-quality complementary and alternative medicine treatments and modalities," Secretary Shalala said. "I look forward to the light he and his colleagues will shed on various alternative approaches to maintaining good health and treating disease."

Dr. Straus has broad basic and clinical research experience related to many diseases for which there are alternative remedies, including chronic fatigue syndrome (CFS), Lyme disease, AIDS/HIV, chronic hepatitis B virus and genital herpes infections, and chronic post-herpetic pain. Dr. Straus is widely regarded for his wide-ranging studies involving patients with CFS, which began in 1979, even before the syndrome was named. These studies have extended from efforts to identify viral etiologies in the syndrome to his more recent immunologic, neuroendocrine and neuropsychologic studies of the disorder. He also has a strong background in investigations of the molecular biology, pathogenesis, treatment, and prevention of human viral infections.

"The American public is increasingly interested in complementary and alternative therapies, and it is critical that NIH put its scientific expertise to work to help determine which therapies are safe and effective," said Harold Varmus, M.D., director of NIH. "The appointment of Dr. Straus, with his experience in alternative therapies and his expertise in clinical evidence, will result in significant expansion of clinical research in this field. He brings to this position a clear sense of leadership, strong management and organizational expertise, and superb communications skills."

Nutraceuticals and Animals

A Veterinarian's View

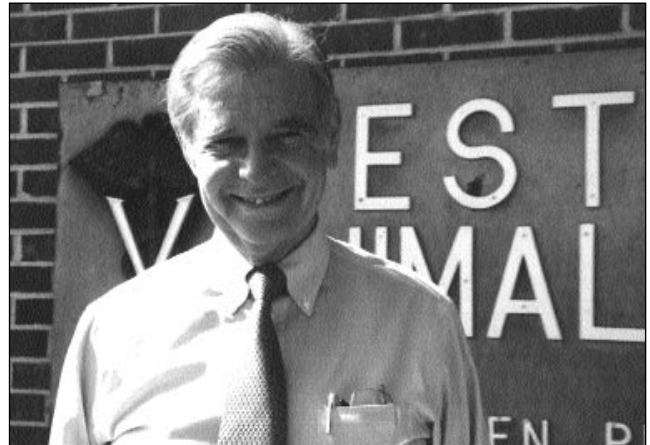
An increasing number of people today are becoming more aware of the important relationship between nutrition and their health. An information-hungry public is ferreting out the latest available literature on the benefits of supplementing our diets with a variety of nutraceuticals, vitamins, minerals, herbs, and antioxidants in an attempt to achieve and maintain optimal health. Just as health care professionals are beginning to take notice of the beneficial effects of supplementation in the adult diet, a growing number of veterinarians are recommending dietary supplements for the animals they treat.

Dr. S. Allen Price has practiced veterinary medicine in Alabama for 48 years. Receiving his DVM from Auburn University, Dr. Price's practice at one time included larger animals such as horses and cattle, but now primarily centers around dogs and cats. He was first introduced to dietary supplements as a result of a personal illness almost 30 years ago.

"I had heard of homeopathy and supplementation therapy back then, but it was definitely more on the fringe at that time," he states. "People knew very little about it, and health care professionals who advocated it were almost ostracized by the traditional medical community. But times have changed. Dietary supplements and homeopathic treatments are more accepted today." In fact the American Veterinary Medical Association is starting to recognize this growing sector of veterinarians.

His interest in the field grew as he reviewed more actual studies pointing to the efficacy of supplementing the diet with naturally occurring substances such as herbs, seeds, flowers, and fruit juices. The library at his clinic in Vestavia Hills, Alabama, just south of Birmingham, is a trove of books, papers, studies, and clinical trials documenting the benefits of supplementation. Seeing the positive results first hand in his personal use of nutraceuticals, he began incorporating them into his treatment of the dogs and cats bought to him by their owners.

"Based on what I've read, seen, and experienced personally," he states, "I felt it was a valid alternative therapy. I use and recommend many of these products in treating a variety of ailments in the animals I see every day." The products that Dr. Price recommends are often easily obtainable, over-the-counter formulations available at health food stores and pharmacies. They range from glucosmine/chondroitin, grape



Dr. S. Allen Price, DVM

seed extract, kava, and echinacea, to olive leaf extract, evening primrose oil, and shark cartilage.

"Animals suffer from many of the same degenerative diseases that affect humans," Dr. Price explains. "Dogs and cats get various forms of cancer, diabetes, and arthritis just like we do. I recommend many supplements for treating these conditions as well as others that have been shown to be helpful in treating everything from depression to psoriasis. Many roots, seeds, and flowers also serve as antioxidants helping to detoxify the animals' bodies."

"We've seen amazing results with many of these natural products," Dr. Price notes. "Adding various supplements to the diet of animals has added pain-free years to their lives. Years ago, people would give these products to their pets, but would not consider taking them themselves. That has changed. I'm seeing an increasing number of people taking more personal control of their own health through supplementation."

For more information, or to find a veterinarian in your area that practices holistic medicine, contact:

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Consumption of *Aphanizomenon flos-aquae* Has Rapid Effects on the Circulation and Function of Immune Cells in Humans

A novel approach to nutritional mobilization of the immune system

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ABSTRACT

Objective: To examine the short-term effects of consumption of a moderate amount (1.5 grams) of the blue-green algae *Aphanizomenon flos-aquae* (AFA), on the immune system.

Methods: Using a crossover placebo-controlled, randomized, double-blinded design, 21 volunteers were studied, including 5 long-term AFA consumers.

Results: Consumption of a moderate amount (1.5 grams) of the blue-green algae *Aphanizomenon flos-aquae* results in rapid changes in immune cell trafficking. Two hours after AFA consumption, a generalized mobilization of lymphocytes and monocytes, but not polymorph nucleated cells was observed. This included increases in CD3+, CD4+, and CD8+ T cell subsets and CD19+ B cells. In addition, the relative proportions and absolute numbers of natural killer (NK) cells were reduced after AFA consumption. No changes were observed in the relative proportions of naïve versus memory T cells, neither in the CD4 or the CD8 fractions. A mild, but significant reduction in phagocytic activity was observed for polymorph nucleated cells. When freshly purified lymphocytes were exposed to AFA extract in vitro, direct activation was not induced, as evaluated by tyrosine phosphorylation and proliferative activity.

Discussion: The changes in immune cell trafficking displayed high degree of cell specificity. Long-term consumers responded stronger, with respect to altered immune cell trafficking. In vitro, AFA did not induce a direct activation of lymphocytes. These data support a signaling pathway from gut-to-CNS-to-lymphoid tissue. The signals from CNS may be crucial for the rapid changes in the general distribution and specific recruitment we observed. Moderate anti-inflammatory modulation may account for the modification of phagocytic activity.

Conclusion: Consumption of AFA leads to rapid changes in immune cell trafficking, but not direct activation of lymphocytes. Thus, AFA increases the immune surveillance without directly stimulating the immune system.

KEYWORDS: (not in title)

Lymphocyte trafficking, natural killer cells, phagocytes.

INTRODUCTION

Blue-green algae are among the most primitive living organisms on Earth. Though they are technically classified as bacteria, they share properties with bacteria and with plants. They contain many biologically active substances that have beneficial effects on human health. Thus, a large research interest in the use of blue-green algae as food supplementation has emerged. Several blue-green algae, including *Aphanizomenon flos aquae* (AFA) have pronounced antibacterial properties,¹ and have protective effects in the classical AMES test.² The blue-green algae *Spirulina* has documented anti-viral^{3,4} and anti-cancer^{5,6} properties. In addition, *Spirulina* subspecies have effects

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on the immune system, by enhancing the phagocytic activity in macrophages^{7,8} inhibiting allergic reactions in rodents,⁹⁻¹¹ and by enhancing antigen-specific antibody production and proliferative responses in chickens.⁸ Other algae contain sulfolipids with potent anti-viral properties.¹² Thus, blue-green algae species contain phytochemicals that are potent modulators of certain immune functions.

The trafficking of immune cells between various locations is an important aspect of the healthy immune system, as part of scavenging for invading pathogens, infected or transformed cells. The various cell types that constitute our immune system are present throughout almost all tissues in our body. The absolute and relative amounts of trafficking immune cells in the blood is rapidly altered in response to chemical messenger molecules. The monitoring of these changes are widely used to evaluate the short-term immune changes to various physical, chemical, and psychological stressors. The various populations of immune cells in normal blood is depicted in Figure 1, along with the surface markers used for their identification.

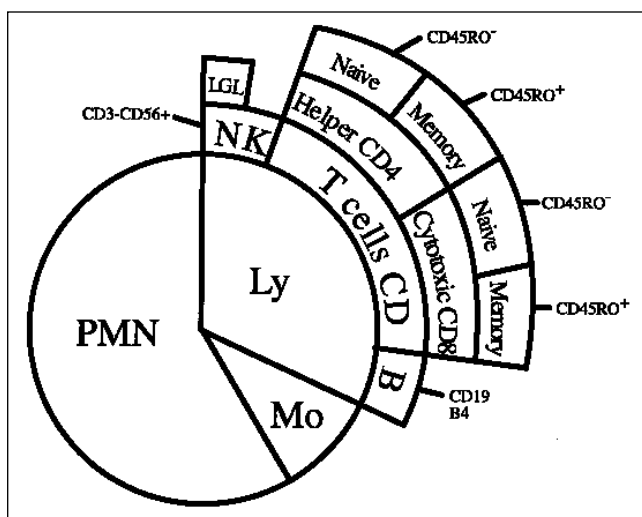


Figure 1: Schematic diagram of the relative proportions of white blood cells and the markers associated with their identification.

Trafficking cells re-circulate between various anatomical locations by entering the blood stream. In order for the cells to exit the blood and enter a new anatomical location, they must be able to adhere and migrate. In almost all tissue (spleen and liver being exceptions), the cells must perform a specific series of tasks in order to transmigrate: 1) Slow down the speed by forming loose adhesion on the vessel wall, and rolling along the endothelial surface, 2) Form a strong adhesion onto the endothelium, and 3) Migrate through the endothelial barrier and the underlying basement membrane.¹³⁻¹⁵ These events are mediated by a combination of chemotactic factors and adhesion molecules. The circulating cells are able to “sense” sites of cellular recruitment via chemokines bound to the endothelium or secreted into

the lumen of the blood vessel. A large number of chemokines are known, and they are able to activate cell subsets in a highly selective manner.¹⁶ Of interest for our data are the chemokines involved in recruitment of natural killer (NK) cells into tissue. Seven out of 8 tested C-C chemokines induced chemotaxis of NK cells,¹⁷ as well as fractalkine.¹⁸ Another chemokine of interest is lymphotactin, which elicits a migratory response in NK and T cells, while having no effect on monocytes and neutrophils.¹⁹ Thus, mechanisms are in place to mediate highly selective patterns of migration and recruitment of specific leukocyte subpopulations.

The re-circulation pattern of immune cells varies in a circadian pattern, which is dependent on neuro-endocrine signals. In one study, a clear circadian rhythm is seen for T cell subsets, but not for NK cells,²⁰ whereas another study reported a clear increase of NK numbers and activity in the morning.²¹ It is believed that high levels of cortisol in the beginning of the day interfere with interleukin-2 production and enhances the migration of lymphocytes from the blood into tissues. Other mechanisms of inducing high levels of cortisol (stress, exercise),²²⁻²⁴ as well as injection of hydrocortisone²⁵ have similar inhibitory effects on lymphocyte migration. Importantly, species variations exist, and stress experiments in rodents cannot directly be compared to human studies. The different physiological responses to various stressors in the human system may be difficult to understand in the light of how apparently similar stressors are perceived in laboratory animals.

The recruitment of NK cells is very sensitive to catecholamines, especially epinephrine.²⁶ The catecholamines have a negative effect on the adhesion of NK cells to the vessel walls, and causes the NK cells to detach. The changes in NK cell trafficking is not accompanied by changes in adhesion molecule expression on the circulating NK cells. The catecholamine-induced accumulation of NK cells in the blood was identical in normal and splenectomized donors, indicating that the spleen was not the relevant reservoir of NK cells.²⁷ Several studies have reported a stress-mediated increase in numbers of B and NK cells in blood.^{28,29}

Throughout the body, many nerve factors are able to function as chemokines, and immune cells express receptors for neurotransmitter molecules. Only some cytokines are regulated by cortisol, and a hierarchy of cortisol-sensitivity has been proposed.³⁰ The bi-directional relationship between neuronal and immunological systems extends to the lymphoid tissues. In addition to the well characterized central nervous system regulation of adrenals, nerve terminals invade all lymphoid tissue, and synapse-like formations can be seen between nerve endings and immune cells in bone marrow, lymph nodes and spleen.³¹ Neuronal control of haematopoiesis has been studied in detail, and a complex feedback system exists, involving multiple cytokines and neurotransmitters.^{32,33} Neuropeptide Y is an

example of a neurotransmitter that is directly able to upregulate adhesion molecules on human endothelial cells.³⁴

The central nervous system regulation of immune surveillance is of functional importance. In mice, when signaling from the sympathetic nervous system to the periphery was interrupted prior to injection of NK-sensitive tumor cells, the numbers of metastases were significantly increased.³⁵ As the NK activity was not altered, nor was the ability to respond to tumor antigens, one possible explanation is that the sympathetic nervous system regulates either NK trafficking or matrix deposition in tissue, thereby regulating the ability of NK cells to migrate to the vicinity of tumors. This was partially confirmed by demonstrating that the sympathetic nervous system modulates lymphocyte recruitment into lymph nodes.³⁶

Consumption of the blue-green algae AFA has increased, and despite a large number of anecdotal reports on health benefits, studies of the exact mechanisms of AFA's effects on immune function were needed. In a previous brief report, we presented preliminary data to show that AFA induced a rapid induction of NK cell recruitment into tissue in humans.³⁷ Since then, we have analyzed the migratory patterns of multiple white blood cell types in a total of 21 study subjects. Upon oral consumption of 1.5 grams AFA, we observed immediate changes in several specific immune parameters.

MATERIALS AND METHODS:

Subjects: Twenty-one non-hospitalized volunteers were analyzed in a double-blinded cross-over fashion, upon informed consent. The volunteers had no known acute or chronic infections. Five were long-term AFA consumers, 2 were occasional AFA consumers, and the remaining 14 had never before consumed AFA. Occasional consumers had previously used AFA daily for at least 6 weeks continuously, but were not consuming AFA regularly during the weeks leading up to this study. No volunteer had taken AFA for at least 24 hours prior to being studied. Ten volunteers were male, and eleven were female. The age range was 20-52 years.

Study design: Each volunteer was studied on two separate days. Any volunteer was always studied at the same time on the two study days, to eliminate the circadian influence on the data. The volunteers were asked to consume the same breakfasts at the same times on the two study days, and not to consume any other vitamin preparations or nutraceuticals for at least 12 hours before the study. The volunteers were required to sit quiet for 45 minutes prior to study start, so that any prior walking or other exercise did not affect the relative proportions of leukocytes. The first blood sample was taken, and the substance was given. Until the sampling of the second blood sample 2 hours later, the volunteer was required to remain quiet and avoid any extensive walking.

Consumables and reagents: Both AFA and placebo were provided by Cell Tech (Klamath Falls, Oregon). The dose given to the volunteers was 1.5 grams, which is the recommended dose for daily supplementation. A list of monoclonal antibodies used for immunostaining and flow cytometry is listed in Table 1.

CD#	Clone	Specificity	Source
CD3	SK7	TCR complex	Becton-Dickinson
CD4	SK3	helper/inducer T cells	Becton-Dickinson
CD8	SK1	cytotoxic T cells	Becton-Dickinson
CD11a	25.3	Alpha-L chain (Beta-2 integrin)	Immunotech
CD11b	D12	Alpha-M chain (Beta-2 integrin)	Becton-Dickinson
CD14	MoP9	PI-anchored receptor, binds LPS	Becton-Dickinson
CD18	7E4	Beta-2 subunit (Integrin)	Immunotech
CD19	89B (B4)B	cell surface molecule	Coulter
CD29	3S3	Beta-1 subunit (Integrin)	Serotec
CD44	F1044-2	H-CAM pgp-1	Serotec
CD49d	L25	Alpha-(VLA)-4 chain (integrin)	Becton-Dickinson
CD62L	TQ1	L-selectin	Coulter

Table 1: List of monoclonal antibodies used in this study.

Purification of mononuclear cells: Fourteen ml of heparinized or EDTA blood was drawn from a peripheral vein. The blood was layered onto a Ficoll gradient and centrifuged to purify the peripheral blood mononuclear cells. Cells were washed, and used for direct immunofluorescence labeling. Samples were fixed in 1% formalin and stored cold and dark prior to flow cytometric analysis.

Flow cytometry: Data were acquired and stored on list mode for subsequent data analysis. The CellQuest software (Becton Dickinson) was used for acquisition and analysis. During analysis, electronic gating was used to eliminate red cells and clumps from the analysis.

Data analysis: The relative proportions of monocytes, B and T lymphocytes and T cell subsets were calculated based on positivity for the MoAbs listed in Table 1. The relative proportion of NK cells was calculated by excluding monocytes and large granular cells from the analysis, then excluding the CD3+ cells, and evaluating the proportion of CD56+ cells in the sample. The number of CD3-CD56+ small lymphocytes was then related to the total number of PBMC. Changes were calculated by comparing the AFA- and placebo-induced values for each volunteer. Figure 1 gives a representation of the various cell types tested, their relationship and the marker used for quantification. By combining the relative proportions with actual cell counts, the absolute numbers of peripheral blood mononuclear cells and PMNs were calculated in 12 volunteers. Also, the changes in absolute numbers of the following subpopulations were calculated: monocytes, CD3+ T

cells, CD19+ B cells, CD4+CD45R0-/+ and CD8+CD45R0-/+ subsets.

Purification of neutrophils: Seven ml of heparinized whole blood was mixed with 1.5 ml of 6% dextran70 in 0.9% saline at room temperature. Sedimentation was allowed for 1 hour. The leukocyte rich supernatant was harvested and the cells pelleted by centrifugation. The pellet was resuspended in 2 ml phosphate buffered saline, which was then layered on top of 3 ml of Ficoll-Hypaque. Gradient centrifugation was performed, and the pellet was resuspended in 0.5 ml of phosphate buffered saline. The remaining red blood cells were lysed by hypotonic shock for 25 seconds, after which isotonicity was restored. Cells were washed, resuspended in RPMI, and kept on ice until use.

Assay for PMN phagocytic activity: The ability of PMN cells to kill *Staphylococcus Aureus* bacteria was performed as follows: *S. Aureus* (frozen aliquots) were defrosted and washed. The bacteria were then opsonized with pooled human serum for 30 minutes in a 37°C shaking water bath. PMN cells and bacteria were added to a series of tubes, and incubated in a 37°C shaking water bath. At the following time points: 5, 15, 30, and 45 minutes a tube was removed, immediately placed on ice, and 0.5 ml icecold serum added in order to stop further phagocytic activity. The tubes were centrifuged in the cold for 5 minutes at 3000 rpm, and the supernatant was decanted. The pellet was stained with Acridine Orange (14.4mg/L) for 1 minute. One ml of icecold buffer was added, and cells were washed 3 times. Cells were resuspended in cold buffer and kept on ice until microscopic examination. A wet mount slide was prepared from each tube for examination in a UV microscope at 100 times magnification. The proportion of phagocytic PMN were evaluated by counting 100 PMN, and counting how many of these cells contained at least 3 bacteria (whether bacteria were live or dead). During the examination, the total number of live versus dead bacteria was counted in 50 PMN.

Statistical analysis: Standard statistical analysis was performed using NNCS software. Paired t-test was used to determine statistical significance. Values that were outside two interquartile ranges from the 25th and 75th percentiles were considered extreme outliers and were removed from the analysis. The removal of outliers did not change the actual conclusion.

RESULTS. Immediate mobilization of mononuclear cells into the blood: The absolute cell counts before and after consumption of either AFA or placebo were monitored in 12 volunteers. The consumption of AFA resulted in increased blood cell counts when compared to placebo. The polymorph nucleated cell (PMN) population did not change, whereas the lymphocyte (Ly) and monocyte (CD14) subsets increased (Figure 2A). Within the lymphocyte subpopulation, the increase was observed in all of the following T cell subsets: CD3+, CD4+, CD8+, as well as in

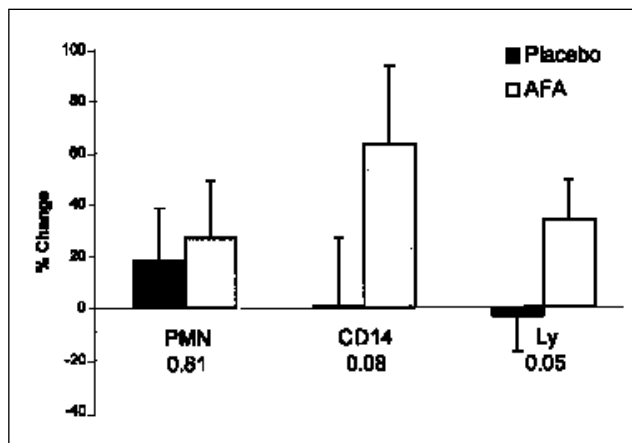


Figure 2A: AFA-induced changes in blood leukocyte populations. The histogram shows the % change of polymorph nucleated cells (PMN), monocytes (CD14), and lymphocytes (Ly). Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

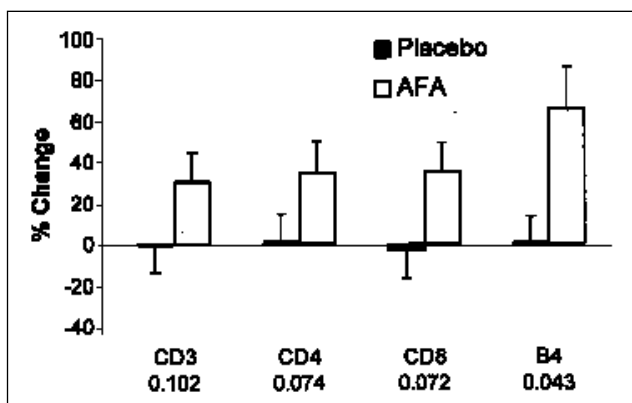


Figure 2B: AFA-induced changes in lymphocyte sub-populations. The histogram shows the % change of total T cells (CD3), T cell subsets (CD4, CD8), and B cell (CD19) lymphocyte populations. Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

the CD19+ B cell population (Figure 2B).

The relative proportions between naïve (CD45A+) and memory (CD45R0+) T cells was monitored in all 21 subjects, for both the CD4+ helper and CD8+ cytotoxic T cell subsets. Despite a tendency for a shift towards less naïve and more memory T cells in the blood, no significant changes were seen in naïve versus memory T cell subsets.

Specific recruitment of CD3- CD56+ small lymphocytes from the blood: In all 21 study subjects, the proportional changes of NK cells was examined. Two hours after AFA consumption, the relative proportion of CD3- CD56+ natural killer cells was decreased, when compared to placebo ($p < 0.03$). The effect was specific for small NK cells

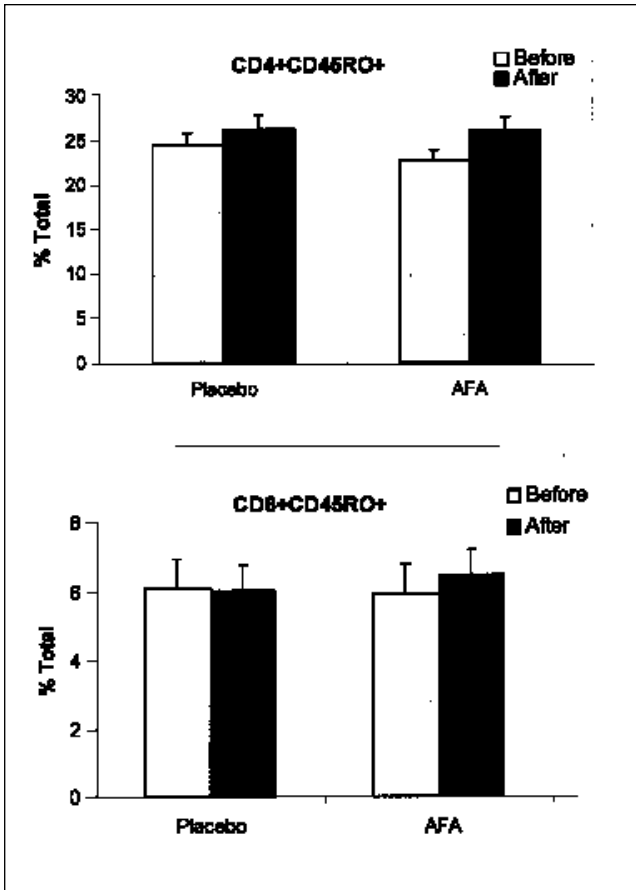


Figure 3: The relative changes in subpopulations of T cells is shown (mean and SEM for 21 volunteers). The helper (CD4+) T cell and cytotoxic (CD8+) T cell populations only showed a slight shift towards less naive and more activated/memory T cells in the circulation, and no statistical significance was reached.

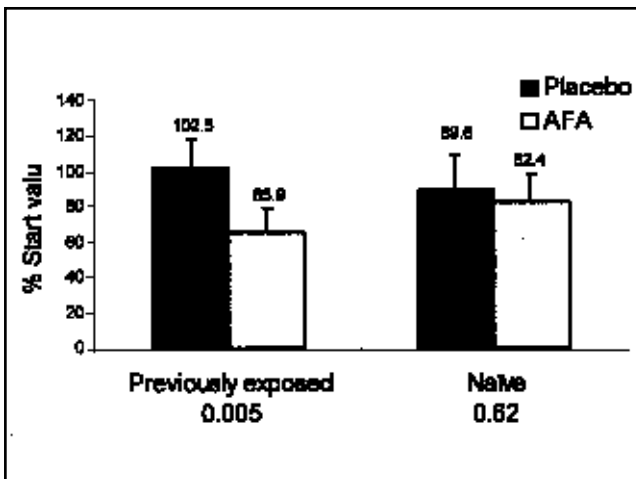


Figure 4: Changes in natural killer cells (NK cells) in % of the starting value. Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

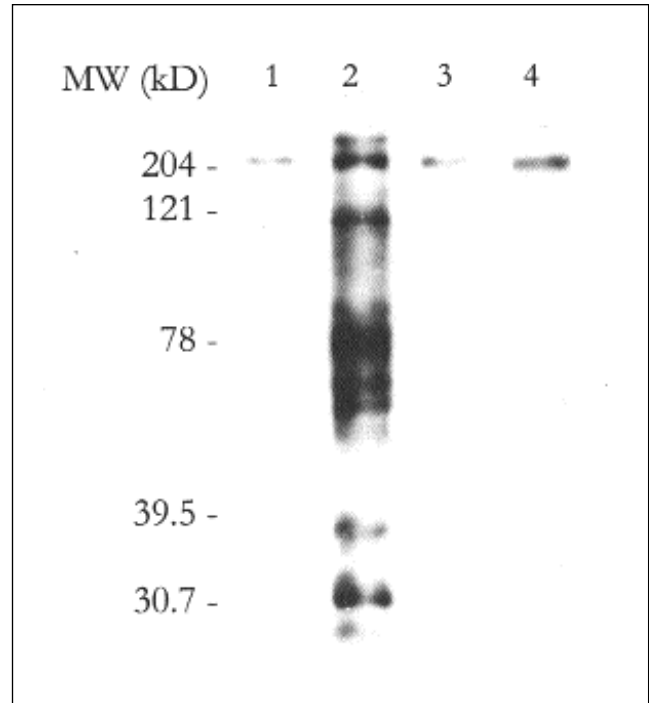


Figure 5: Western blotting of tyrosine phosphorylation of proteins extracted from unstimulated lymphocytes (lane 1) versus lymphocytes incubated with Pokeweed Mitogen (PWM, positive control, lane 2) or AFA (lane 3: extract 1:5, lane 4: extract 1:25). Incubation of freshly purified human lymphocytes with AFA extract did not induce tyrosine phosphorylation. The data are representative of 4 similar experiments.

(low forward/side scatter properties), as the subset of cells defined as large granular lymphocytes (CD14-negative, large granular cells) was not affected (data not shown). Long-term consumers produced a more pronounced response than naive volunteers. When the volunteers were grouped into long-term AFA consumers and naive volunteers, naive volunteers displayed a minor reduction in NK cells after AFA consumption, whereas long-term consumers displayed a pronounced reduction ($p < 0.005$).

Adhesion molecule expression on circulating leukocytes: We examined the expression of a series of adhesion molecules on the surface of monocytes, B and T cells before and after AFA exposure in vivo and in vitro. The following adhesion molecules and subunits were examined: CD62L, CD11a, CD11b, CD18, CD29, CD44, and CD49d. The fluorescence intensity was monitored by % positive, as well as mean and median fluorescence values. Short-term incubation (90 minutes) in vitro with AFA extract resulted in a moderate loss of CD62L on B as well as T cells, and a weak upregulation of CD11b, but no other changes in the expression of the following adhesion molecules: CD11a, CD18, CD29, CD44, and CD49d. Analysis of adhesion molecule expression on lymphocytes from volunteers 2 hours post AFA consumption showed moderate changes in CD62L expression, but no other changes (data not shown).

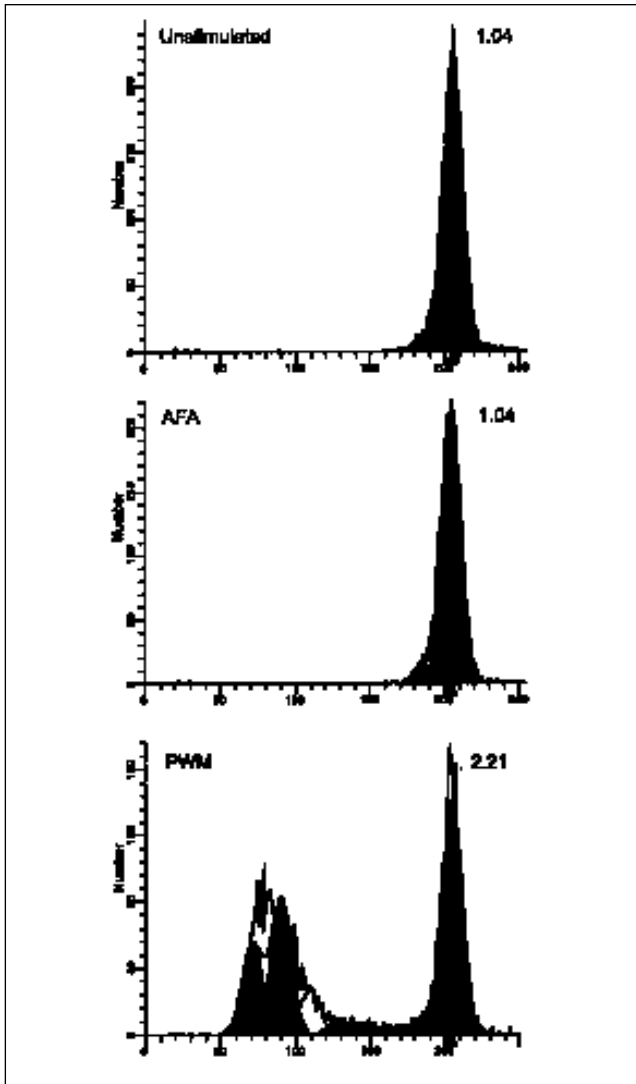


Figure 6: Flow cytometric evaluation of lymphocyte proliferation after 5 days of culture with no stimulation (top), with AFA water extract (middle), and Pokeweed Mitogen (PWM, bottom). The X axis displays fluorescence intensity, where loss of fluorescence corresponds to proliferative activity. The proliferative indexes for each culture condition is displayed in upper right corner of each histogram. The experiment was conducted three times, where all cultures were performed in triplicate.

AFA extract does not activate lymphocytes directly: We tested whether AFA extract could directly activate lymphocytes in vitro. When purified mononuclear cells were incubated with AFA extract, no activation was seen, as examined by tyrosine phosphorylation after 1-20 minutes of AFA exposure (Figure 5) and proliferative responses after 5 days of AFA exposure in vitro (Figure 6).

Modulation of the phagocytic activity of polymorph nucleated (PMN) cells: The phagocytic activity of PMN cells was evaluated, using PMNs from blood samples drawn before and 2 hours after AFA consumption. The

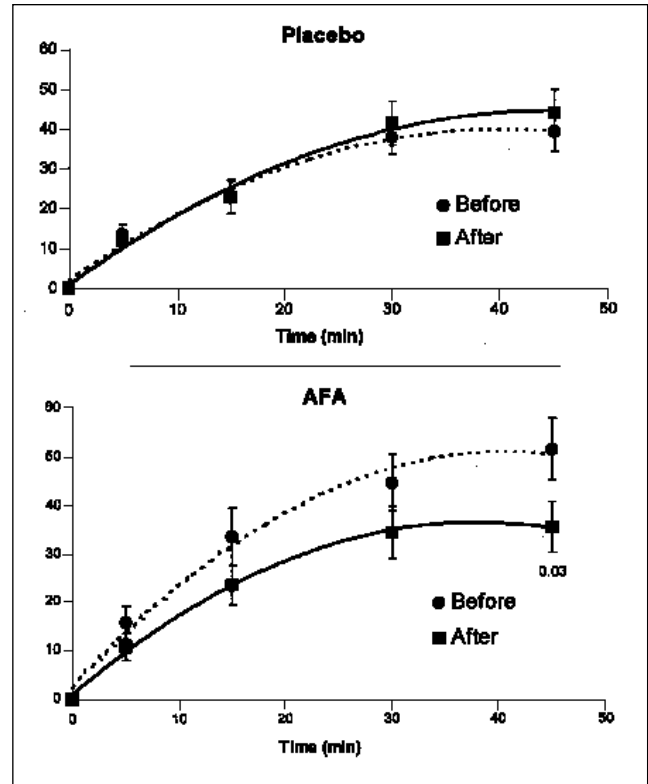


Figure 7: Phagocytic activity of polymorph nucleated cells (PMN) from volunteers before and after placebo or AFA ingestion. The phagocytic activity was unaffected by placebo, but was moderately reduced by AFA, thus resulting in a lower maximum phagocytic capacity, and a lower phagocytic rate.

phagocytic activity was monitored at different times of incubation. When the study subjects had ingested placebo, no differences on phagocytic activity was seen. In contrast, after consumption of AFA, a mild decrease in phagocytic activity was measured (Figure 7). This effect only reached levels of significance at longer incubation times (see legend to Figure 7).

DISCUSSION

Based on the many case reports on beneficial neurological and immunological effects of consumption of the blue-green algae *Aphanizomenon flos-aquae*, we studied the immune activation within 2 hours after ingestion of 1.5 grams AFA. This dose is recommended for food supplementation. We examined several aspects of immune cell migration and function. The data presented in this paper indicate a mild, but consistent effect on the human immune system.

The absolute numbers of circulating leukocyte subsets was increased. This effect was limited to lymphocytes and monocytes, whereas polymorph nucleated cells were not affected. This indicates a selective mobilization of lymphocytes and monocytes from primary or secondary lym-

phoid tissues, into the blood circulation. Thus, more monocytes, B and T cells were released into the blood. In the initial preliminary study (involving 1 occasional and 4 regular AFA consumers), AFA consumption induced a substantial transient recruitment of NK cells in all five volunteers, peaking at 2 hours and rapidly declining.³⁷ In the current analysis of 21 people, there was a specific recruitment from the blood of small NK cells. It could be argued that AFA only leads to margination (i.e. lymphocytes sticking to the vessel walls without transmigration). However, margination is not a permanent phenomenon, and the on/off rate would allow us to sample some cells that have margined and later released from the blood vessel wall. Such cells would likely demonstrate altered adhesion profiles, which we did not find. In addition, as the recruitment of cells from circulation into lymphoid tissue is highly cell type specific, mediated in part by cell-type specific chemokines, transmigration would provide a more plausible explanation.

Increase in adhesion molecule expression was previously observed in a small number of long term consumers.³⁷ The present study reports data from a more thorough evaluation. When examining the profile of adhesion molecules on the surface of circulating lymphocyte subsets, we found occasional shifts in adhesion molecule expression, confirming earlier observations, but in this larger study we found no consistent differences induced by AFA in vivo. This evaluation is hampered by the fact that we are not able to directly sample the cells that have left the circulation as a result of AFA. Thus, AFA did not uniformly affect the adhesion profile of all circulating lymphocytes.

The low dose of AFA ingested and the rapidity of the observed effects do not support a direct effect, where bioactive molecules in AFA would be absorbed into the blood, and transported to the bone marrow and spleen, and there result in cellular changes leading to release of cells into the blood. A more plausible model for explanation is that neuro- or immune- active substances in AFA leads to triggering of a gut-to-brain activation. It has been reported that IL-1 beta is able to mediate a gut-to-brain communication via the abdominal vagus nerve.^{38,39} Thus, in terms of rapid modulation of leukocyte re-circulation, a gut-to-brain signal would result in brain-to-lymphoid tissue signals, including the rapid release of chemokines. Many neuropeptides are either chemotactic or immuno-modulatory. As nerve terminals wrap around the high endothelial regions of lymphocyte recruitment in the peripheral tissue, a central activation could rapidly amplify and alter cellular recruitment in a highly selective manner. In the bone marrow, nerve terminals come in close contact with developing and maturing cells, and could regulate the volume of cells released into the blood circulation.

The rapid changes in leukocyte re-circulation were stronger in long-term AFA consumers. Since the study design was double-blinded and randomized, the volunteers

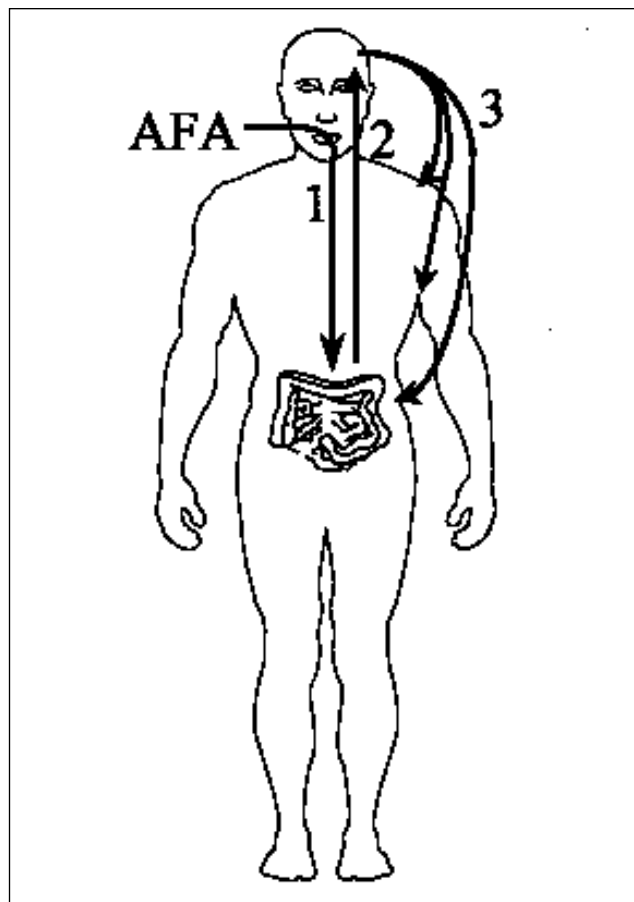


Figure 8: Hypothetical model for AFA-induced immuno-modulation. 1: Ingestion of AFA, and release of bioactive phytochemicals in the stomach and/or upper intestine. 2: Release of cytokine(s) in the gut trigger vagus nerve signals from gut to CNS. 3: Central nervous system signals to the peripheral lymphoid tissues, resulting in altered immune cell trafficking.

were not themselves aware of when they were receiving AFA versus placebo. Given the suggested CNS-mediated modulation of the immune system, a conditioning may have been established in which the CNS may recognize the stimulation by AFA and in previously exposed consumers add a conditioned component to the immune activation of cell trafficking.

During our studies, we have been on guard for observations that could point in the direction of over-activation of the immune system. More is not always better. An over-activation of the immune system could be associated with circulating immune complexes and increase in inflammatory processes that could be detrimental to health. We found no indications of a direct activation of any component of the immune system or a general activation of the immune system as a whole. The increased trafficking of immune cells should translate into a better immune surveillance, i.e. a better and more efficient patrolling of microbial invaders, as well as virus-infected or transformed cells. We see this

as very positive for a potential use of AFA in various clinical situations or as a nutritional support for the prevention of viral infections. This data also points to further research in a potential role for AFA in cancer prevention.

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Favorable Effects of Blue-Green Algae *Aphanizomenon flos-aquae* on Rat Plasma Lipids

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ABSTRACT

Background: Polyunsaturated fatty acids (PUFA) are essential for human health. There are indications that the lipid fraction of blue-green algae *Aphanizomenon flos-aquae* contains about 50% of PUFA and may be a good dietary source of PUFA. The purpose of this study was to investigate the effect of diets supplemented with algae on blood plasma lipids.

Methods: Rats were fed with four different semisynthetic diets: i) standard, with 5% soybean oil; ii) PUFA-free with 5% coconut oil; iii) PUFA-free with 10% algae; iv) PUFA-free with 15% algae. After 32 days the levels of plasma fatty acids, triglycerides and cholesterol were studied.

Results: Rats fed the PUFA-free diet demonstrated an absence of linolenic acid (LNA) in plasma; however, supplementation with algae resulted in the same level of LNA as controls, an increased levels of eicosapentaenoic acid and docosahexaenoic acid, and a decreased level of arachidonic acid. Dietary supplementation with 10% and 15% algae decreased the plasma cholesterol to 54% and 25% of the control level, respectively ($P < 0.0005$). Plasma triglyceride levels decreased significantly ($P < 0.005$) after diet supplementation with 15% algae.

Conclusion: Algae *Aphanizomenon flos-aquae* is a good source of PUFA and because of potential hypocholesterolemic properties should be a valuable nutritional resource.

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INTRODUCTION

Previous research identified the important role of dietary polyunsaturated fatty acids (PUFA) in human health. A deficiency in n-3 PUFA has been linked to immunosuppression,¹ arthritis,² cardiovascular diseases,³⁻⁶ mental^{7,8} and dermatological⁹ problems. n-3 PUFA in human and animal models have anti-inflammatory activity^{2,10,11} that may be mediated by decreasing arachidonic acid level and thereby suppressing the production of specific cytokines.¹² Furthermore n-3 fatty acids have been shown to decrease certain cancer risks,^{13,14} prevent platelet aggregation^{6,15} and to lower blood cholesterol, possibly by stimulating its excretion into bile.^{3,16}

The North American diet is believed to be deficient in PUFA, especially in n-3 fatty acids.¹⁷ Dietary supplementation with fish oil rich in n-3 eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) has been recommended as a potential treatment for hypercholesterolemia.^{15,18} Much empirical evidence over the past decade suggests that *Aphanizomenon flos-aquae* (*Aph. flos-aquae*), a blue-green alga growing naturally in Upper Klamath Lake, Oregon may be a good dietary source of PUFA. Nearly 50% of the lipid content of dried *Aph. flos-aquae* (5% to 9% of total dry weight) is composed of PUFA, mostly n-3 α -linolenic acid.

In our experiments using rats as the animal model, *Aph. flos-aquae* not only served as source of dietary PUFA but also significantly lowered blood cholesterol and triglyceride levels.

METHODS

Animals: Thirty-two adult male Sprague-Dawley rats were randomly distributed into 4 groups. Animals were placed into individual wire cages, and maintained at 22° C with a 12-hour light-dark cycle. Food and water were supplied *ad libitum*. For 32 days the animals were fed with the following semipurified test diets based on the American

Institute of Nutrition (AIN-76) standard:

1. Standard diet containing 5% soybean oil (SBO);
2. PUFA-deficient diet containing 5% coconut oil (PUFA-D);
3. PUFA-deficient diet containing 10% algae (Alg10);
4. PUFA-deficient diet containing 15% algae (Alg15).

The algal material used in this study was supplied by Cell Tech (Klamath Falls, OR) and contained 6.3% lipids. Feed was provided by Purina Test Diets (Richmond, IN).

After the feeding trial, the animals were fasted overnight and euthanised by carbon dioxide inhalation. Plasma was collected by heart puncture in a tube containing 100 µl 0.5 M EDTA (pH 8.0), centrifuged at 3,000 g for 15 minutes, and stored at -80°C.

Lipid Analysis: Blood fatty acid analysis was performed using a direct transesterification method¹⁹ as modified by Mosers.²⁰ In brief, 250 µl of plasma was vortexed with 1 ml methanol:methylene chloride (3:1). 50 nmol of 17:0 free fatty acid (internal standard) in 50 µl of hexane was added to this mixture. Under continuous vortexing 200 µl of acetyl chloride was added and the mixture was incubated in the oven at 75°C for one hour. After cooling for 15 min at room temperature 4 ml of 7% potassium carbonate was added, vortexed, and then 2 ml of hexane was added. The mixture was vortexed for 60 sec and then centrifuged at 1750 g for 10 min at 4°C. The hexane layer was removed, 2 ml of acetonitrile was added and the mixture was centrifuged at 1120 g for 5 min at 4°C. The hexane layer was removed, dried under nitrogen to a final volume of approximately 100 µl, and 1 µl of the sample was used for analysis. Fatty acid identification was performed on a Hewlett-Packard 5890 series II model gas chromatograph-mass spectrometer GC-MC with a Hewlett-Packard 5971 mass spectrometer (Hewlett-Packard, Wilmington DE). Soybean and coconut oils were methylated by acid methanolysis before fatty acid analysis. The algae material was soaked in methanol, extracted and then methylated by acid methanolysis prior to fatty acid analysis.

Plasma triglycerides and cholesterol were measured on the automated clinical chemistry analyzer Roche BHO/H917 using corresponding Boehringer Mannheim kits.

Statistics: Statistical difference between groups was determined using unpaired Student's t-test. Difference in fatty acid profiles was evaluated using repeated measures analysis and contrast tests²¹. For all analysis, differences of $p < 0.05$ were considered statistically significant.

RESULTS

Dietary Fatty Acids: Fatty acid composition of *Aph. flos-aquae*, soybean oil and coconut oil used in this study is represented in Table 1. The composition of soybean and coconut oil in the present study is close to that found in the

TABLE 1
Fatty acid composition (% of total fatty acids)
of soybean oil, coconut oil, and algae

Fatty Acid	Source of Fatty Acids		
	Soybean oil	Coconut oil	Algae
Caprylic (8:0)	-	9.70	-
Capryc (10:0)	-	7.50	-
Lauric (12:0)	-	42.10	-
Myristic (14:0)	-	22.40	9.10
Palmitic (16:0)	14.69	18.20	36.60
Palmitoleic (16:1)	-	-	11.90
Margaric (17:0)	-	-	0.89
Stearic (18:0)	5.40	-	2.70
Oleic (18:1)	26.80	-	6.70
Linoleic (18:2n-6)	44.40	-	7.40
Linolenic (18:3n-3)	8.00	-	22.30
Arachidic (20:0)	0.35	0.14	-
Arachidonic (20:4n-6)	-	-	0.65
Eicosapentaenoic (20:5n-3)	-	-	0.08
Behenic (22:0)	0.33	-	-
Total polyunsaturated	52.40	-	30.43
Total saturated	20.77	100.04	49.29

Table 2
Lipid composition (%) of experimental diets

Indices	Diets			
	SBO	PUFA-D	Alg10	Alg15
Oil Source				
Soybean oil	5.00	0.00	0.00	0.00
Coconut oil	0.00	5.00	4.50	4.250
Algae	0.00	0.00	10.00	15.00
Total fat	5.00	5.00	5.13	5.20
Fatty Acid Content				
Linoleic acid (18:2n-6)	2.22	0.00	0.05	0.07
Linolenic acid (18:3n-3)	0.40	0.00	0.14	0.21
Total polyunsaturated (PUFA)	2.62	0.00	0.19	0.28
Lauric acid (12:0)	0.00	2.11	1.89	1.79
Myristic acid (14:0)	0.00	1.12	1.07	1.04
Palmitic acid (16:0)	0.73	0.91	1.05	1.12
Stearic acid (18:0)	0.27	0.00	0.02	0.03
Oleic acid (18:1)	1.34	0.00	0.04	0.06
Total saturated (SFA)	1.00	4.14	4.03	3.95
PUFA/SFA	2.62	0.00	0.05	0.07
n-6/n-3	5.55	-	0.36	0.36

literature.²² Soybean oil is rich in linoleic acid (LA, 18:2n-6; 44.4% of total lipids) and contains a substantial amount of α -linolenic acid (LNA, 18:3n-3; 8%). *Aph. flos-aquae* is richer in LNA (22.3%) and contains less LA (7.4%) than soybean oil. *Aph. flos-aquae* has also small amount (0.65%) of arachidonic acid (AA, 20:4n-6) and traces (0.08%) of EPA (20:5n-3). Coconut oil is free of both n-3 and n-6 fatty acids.

Fatty acid composition of the various diets is represented in Table 2. SBO and PUFA-D diets had a total of 5% lipids provided by soybean and coconut oils. Because of a slightly higher amount of lipids in algae (6.29%) than expected (5%), Alg10 and Alg15 diets contained correspondingly 5.13% and 5.20% of lipids. Ratios of PUFA to saturated fatty acids (SFA) and n-6 to n-3 varied considerably between the diets.

Calculations showed that *Aph. flos-aquae* contains 1.40% LNA and 0.46% LA of total algal dry weight. Diets containing 10% and 15% of algae (corresponds to 0.63% and 0.94% of algal lipids) provided a total dietary intake of 0.14% LNA and 0.047% LA for the Alg10 diet, and 0.21% LNA and 0.07% LA for the Alg15 diet (Table 2). Therefore, amounts of n-3 and n-6 PUFA in algae-supplemented diets

were significantly lower than in the positive SBO control diet. The SBO diet contained 2.9 times more LNA and 44 times more LA than the Alg10 diet, and 1.9 times more LNA and 32 times more LA than the Alg15 diet. Furthermore, the n-6/n-3 ratio varied significantly between the algal (0.36) and the SBO (5.55) diets.

Because of the high SFA content in coconut oil, algae supplemented diets contained more SFA than the SBO diet (Table 2). Alg10 and Alg15 diets contained four times more SFA than the SBO diet. Therefore, the PUFA/SFA ratio was significantly lower in the Alg10 (0.05) and Alg15 (0.07) diets compared to the SBO (2.62) diet. The main SFA in the Alg10 and Alg15 diets were lauric acid (12:0, ~1.84% of total diet), myristic acid (14:0, ~1.06%) and palmitic acid (16:0, ~1.08%).

Plasma Fatty Acids: Figure 1 shows a full rat plasma fatty acids profile. Plasma palmitate levels increased with palmitate dietary intake ($r=0.60$), reaching the highest level in the Alg15 group ($p<0.01$). Plasma LA increased with dietary LA intake ($r=0.67$), being highest in the SBO group ($p<0.001$), which correlates with the high amount of LA in this diet. In rats fed coconut oil deficient in LA, the plasma LA level was 36% ($p<0.0005$) of the SBO control level.

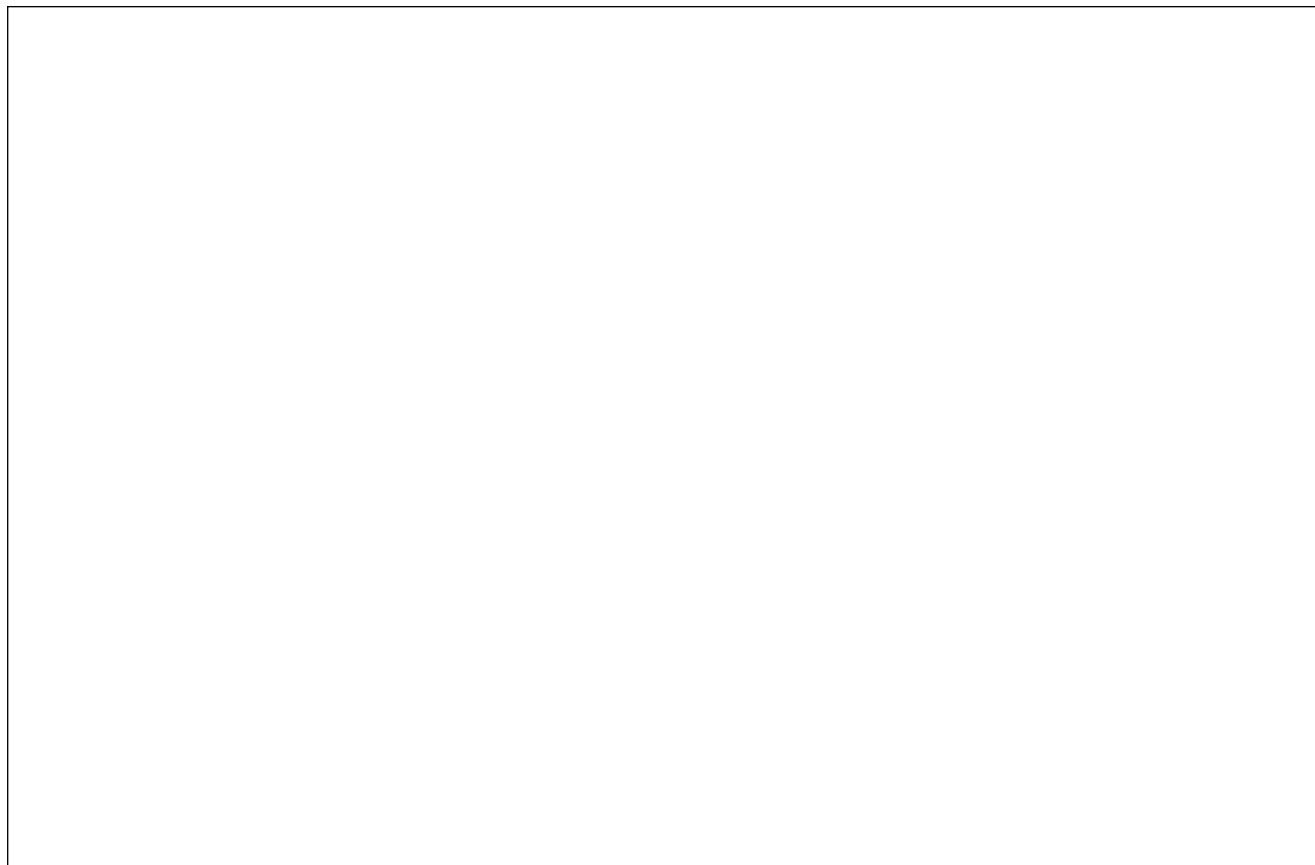


FIGURE 1. Plasma fatty acids profiles in animals fed different diets (Mean±SEM).

In PUFA-D animals diet supplementation with algae increased plasma oleic acid and LA levels but decreased AA level.

When the PUFA-D diet was supplemented with *Aph. flos-aquae*, plasma LA level was restored to 71% (Alg10) and 67% (Alg15) of the SBO level, in spite of the fact that algae supplemented diets contained less than 3% the amount of LA present in the control SBO diet.

Plasma arachidonic acid (AA, 20:4n-6) also decreased with increasing AA dietary intake ($r=-0.88$) and was highest in the plasma of the SBO group ($p<0.001$). However, the plasma AA level correlated positively with the dietary level of the AA precursor LA ($r=0.64$), which was the highest in the SBO diet. Rats fed the PUFA-D diet, which contains no LA, had a plasma AA level significantly lower than controls fed the SBO diet ($p<0.01$). However, supplementing PUFA-D diet with *Aph. flos-aquae* further decreased plasma AA levels in a dose-dependent manner, in spite of the low LA and AA content in Alg10 and Alg15 diets.

In order to better appreciate the variation in plasma PUFA levels, Figure 2 shows the plasma lipid profile for some PUFAs on a different scale than Figure 1. Rats fed the PUFA-D diet had no plasma LNA, which is consistent with the absence of LNA in coconut oil. However, algae supplementation of the PUFA-D diet restored plasma LNA to

the SBO (control) level, in spite of the fact that the algal diets contained only 35% (Alg10) and 52% (Alg15) of the LNA present in the SBO diet.

Feeding rats the PUFA-D diet increased plasma diho-mo- α -linolenic acid (DGLA, 20:3n-6) 5 times above the SBO control level ($p<0.05$). Algae supplementation of PUFA-D diet decreased plasma DGLA level in a dose-dependent manner. Levels found in the algae-treated animals were still higher than SBO controls, though this difference was not statistically significant ($p<0.09$).

EPA was absent in the plasma of rats fed the PUFA-D diet. However, when the diet was supplemented with 10% and 15% algae, plasma EPA increased 6 times ($p<0.005$) and 1.7 times ($p>0.1$) above the SBO control level, respectively. The DHA (22:6n-3) concentration in the plasma of rats fed the PUFA-D diet was 35% lower than in controls, although this difference did not reach statistical significance. Supplementation with 10% algae increased the plasma DHA level by a factor of 2 ($p<0.05$), but supplementation with 15% algae did not affect the plasma DHA level. The effect of algae on EPA and DHA levels in rat blood plasma may not be dose-dependent.

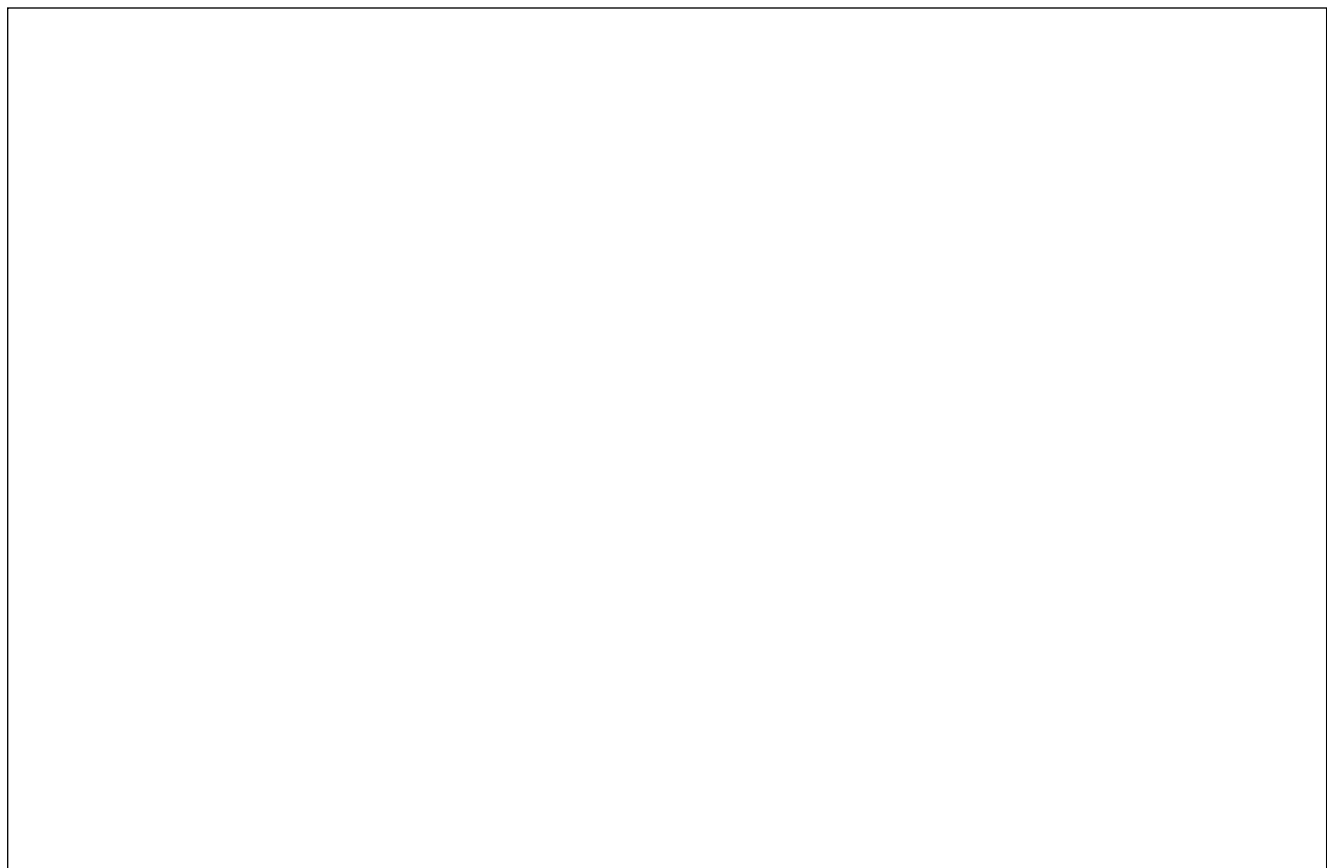


FIGURE 2. Specific PUFA profile in rats plasma (Mean±SEM).

Rats fed PUFA-D diet demonstrated an absence of LNA in plasma, however, animals fed Alg10 and Alg15 diets had the same level of LNA as controls, and increased EPA and DHA levels.

Thus, supplementation of the PUFA-D diet with algae normalizes fatty acid levels in plasma of PUFA deficient animals and makes their PUFA profile similar to controls.

Plasma Triglycerides and Cholesterol: Algae affected not only free fatty acids but also other lipids in the blood. The PUFA-D diet did not significantly decrease plasma triglycerides level relative to SBO controls (Figure 3). However, supplementation of the PUFA-D diet with 15% algae decreased plasma triglycerides to 24% of the SBO control level ($p < 0.005$). The PUFA-D diet supplemented with 10% algae did not affect significantly the plasma triglycerides level. Levels of triglycerides in blood plasma positively correlated with PUFA/SFA ratio ($r = 0.87$).

Cholesterol concentration in plasma was very sensitive to diet supplementation with algae (Figure 3). Rats fed the PUFA-D diet had a lower cholesterol level ($p < 0.05$) than the SBO controls. The PUFA-D diet supplemented with algae caused a further dose-dependent decrease in the plasma cholesterol level. Supplementation with 10% and 15% algae decreased the plasma cholesterol level to 54% and 25% of the SBO control level ($p < 0.0005$), respectively. Cholesterol

and triglyceride levels were positively correlated ($r = 0.91$).

Cholesterol levels also positively correlated to plasma PUFA/SFA ratio ($r = 0.81$) and to plasma stearic acid ($r = 0.86$). On the other hand, blood cholesterol was strongly negatively correlated with plasma myristic acid ($r = -0.99$). From a dietary standpoint, blood cholesterol was related only to dietary palmitic acid ($r = -0.95$).

DISCUSSION

The results reported here demonstrate that, in the rat model, *Aph. flos-aquae* appears to be a good source of PUFA. Calculations showed a good correlation between dietary and serum levels of LA. However, the correlation between dietary and serum LNA was poor. Rats fed the PUFA-deficient diet supplemented with *Aph. flos-aquae* had blood levels of LNA comparable to levels found in rats fed soybean oil diet containing nearly three times the amount of LNA. This suggests a higher bioavailability of LNA in *Aph. flos-aquae* compared to soybean oil. Furthermore, in spite of the fact that blood levels of LNA

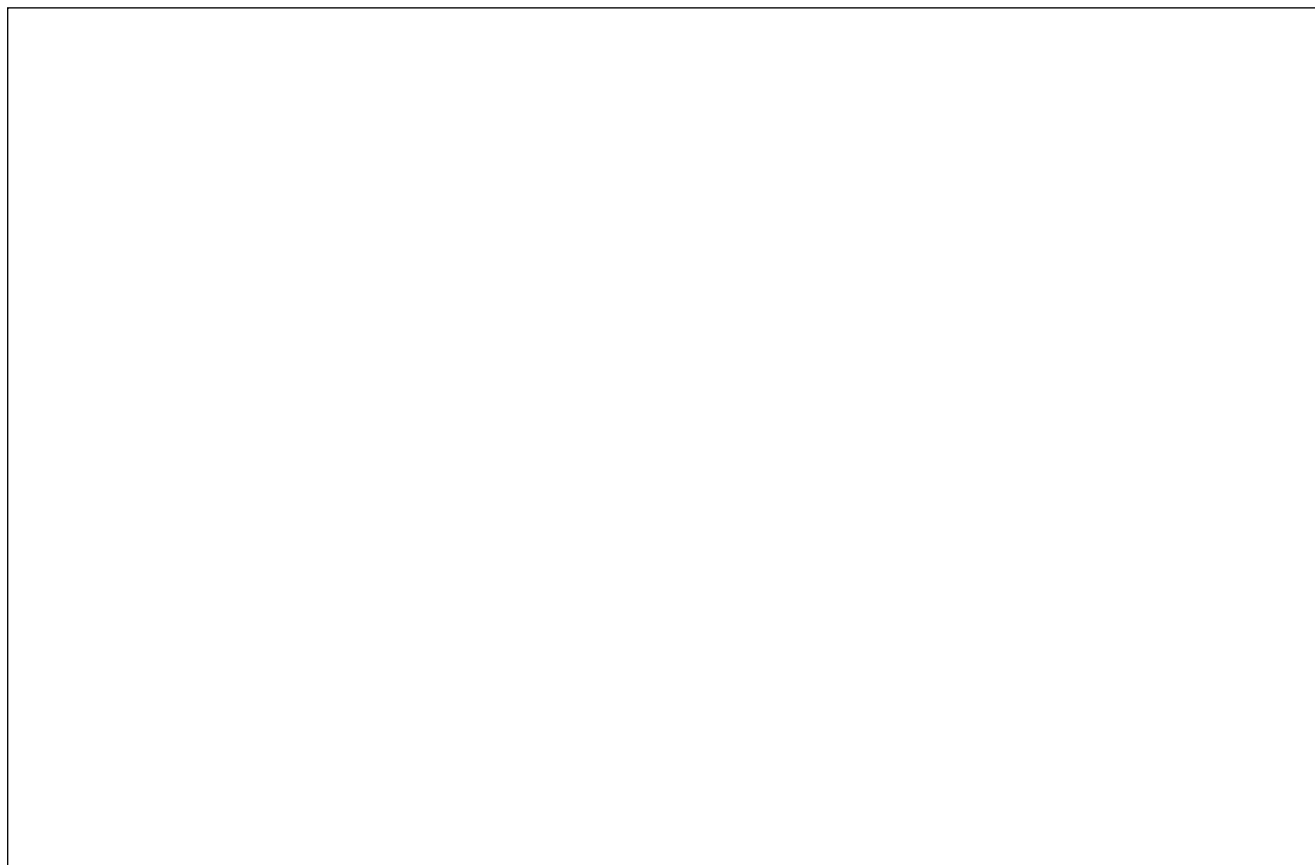


FIGURE 3. Lipid levels and PUFA/SFA ratio in rats fed different diets (Mean \pm SEM). * $P < 0.01$. PUFA-D diet supplemented with algae decreased triglycerides and cholesterol levels in rat blood plasma.

were similar in rats fed SBO and algae supplemented diets, there were significantly higher blood levels of EPA in the rats fed the *Aph. flos-aquae* diet. It has been previously suggested that increased dietary SFA increased the rate of conversion of LNA to EPA, whereas increased dietary n-6 PUFA decreased this conversion by 40-50%.²³ This dual effect could explain the fact that rats fed algae supplemented diets, which contained significantly more SFA, had higher blood levels of EPA than rats fed the SBO diet, which contained significantly more LA.

When the two main plasma n-6 PUFA (LA and AA) were analysed as profile, there was a very good positive correlation between LA dietary intake and the total level of n-6 PUFA. However, the n-6 PUFA profiles in rat plasma were different between the various groups. Supplementing diets with algae led to a dose-dependent decrease in plasma AA and concomitant accumulation of LA. This could be due to *Aph. flos-aquae's* content of phycocyanin. Phycocyanin, the blue pigment in blue-green algae, was recently shown to have significant anti-inflammatory properties^{24,25} which seemed to be mediated by an inhibition AA metabolism.²⁶ The presence of phycocyanin in the algae supplemented diets may have inhibited AA synthesis and consequently promoted the accumulation of LA.

This study suggests that *Aph. flos-aquae* has significant hypocholesterolemic properties when compared to soybean oil. Many studies have demonstrated the hypocholesterolemic properties of n-3 PUFAs^{16,27,28} and the negative correlation between PUFA/SFA ratio and blood cholesterol levels.^{29,30} In this study, cholesterol levels were positively correlated with the PUFA/SFA ratio. The main SFA present in the diet of the algae-treated groups were lauric, myristic and palmitic acids, which were all demonstrated to promote hypercholesterolemia to some degree.³¹⁻³³ This suggests that the hypocholesterolemic effect of *Aph. flos-aquae* is likely to be mediated by factors other than its fatty acid content. Specifically *Aph. flos-aquae* contains a significant amount of chlorophyll (1-2% dry weight) which was shown to stimulate liver function, and increase bile secretion³⁴. A synthetic derivative of chlorophyll was shown to reduce blood cholesterol.³⁵ Therefore, it is possible that *Aph. flos-aquae* chlorophyll is responsible for the increased liver function and secretion of cholesterol into bile. *Spirulina*, another blue-green algae, was also shown to affect cholesterol metabolism by increasing HDL levels.³⁶ According to other sources³⁷, hypocholesterolemic effect of blue-green algae (*Nostoc commune*) is related to their fibers.

In conclusion, this study demonstrated that *Aph. flos-aquae* is a good source of PUFA with strong hypocholesterolemic properties. *Aph. flos-aquae's* ability to increase serum level of LNA, EPA, DHA, and lower level of AA in rats makes it a good candidate for future nutritional research in humans.

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