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Pilot Study: An Emulsified Fish Oil Supplement Significantly Improved C-Reactive Protein, Hemoglobin, Albumin and Urine Output in Chronic Hemodialysis Volunteers
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The Journal of the American Nutraceutical Association (JANA) is a peer-reviewed journal whose editorial board is composed of physicians, pharmacists, academicians, and researchers striving to educate healthcare professionals and consumers about the latest research in the growing field of nutraceutical science.

In the January 2002 issue of Consumer Reports, an article titled "Joint Remedies" provides guidance to consumers on nutraceutical products that contain glucosamine and chondroitin. While we applaud the efforts of Consumer Reports to differentiate glucosamine and chondroitin supplements on the basis of their labeled claims and cost to consumers, we take exception to the concluding statement in this article that advises consumers: "While no one knows which formulation works best, it makes sense to try one of the least expensive combination products." In essence, this recommendation by a highly respected publication guided the consumer in their purchasing decisions exclusively on the price of products that matched label claim. While price and content of the product as compared to label claim are important issues, they are not enough, for it is equally important to evaluate bioavailability, which in turn can impact the efficacy of these products, especially those containing chondroitin.

Taber’s Medical Dictionary defines bioavailability as "the rate and extent to which an active drug or metabolite enters the general circulation, permitting access to the site of action." Bioavailability is determined either by measurement of the concentration of the ingredient in body fluids, or by the magnitude of the pharmacologic response. Simply put, when a product is not absorbed by the body, it will not produce the desired effect.

As members of the editorial board of JANA, we are concerned that the approach to product selection suggested in this Consumer Reports article does not adequately address the complex issue of bioavailability. We agree that a product must contain the amounts stated on the label. However, the bioavailability and "efficacy" of a nutrient is in many cases dependent on more than the quantity of the ingredient found in the tablet or capsule. This is an important consideration, for the level of bioavailability can affect the cost of the compound. This is especially true for chondroitin sulfate, as discussed in our journal in a study that evaluated the potential intestinal transport of several marketed sources of chondroitin sulfate. In an original research study published in the Journal of the American Nutraceutical Association (Vol. 3, No. 1, 2000, 37-44), Adebowale and colleagues at the University of Maryland School of Pharmacy reported that the permeability coefficient for transport of chondroitin sulfate is affected by the molecular weight of the raw materials used, demonstrating that the bioavailability can be related to the size of the molecule. Several products examined in this study that passed "label claim" failed in an absorption model. This means that although a product meets its labeled claim, its molecular weight, size, or configuration can impact its bioavailability, with little or none of the ingredients actually absorbed, negatively impacting the product’s intended clinical effect.

Well-researched parameters that can affect bioavailability include molecular size, shape, and weight, excipients in the formulation, and the manufacturing process.

We have all heard of tablets compressed so hard that they do not dissolve in the GI tract, and therefore have no bioavailability even when they meet label claims for quantity. The University of Maryland study concluded that the bioavailability of chondroitin sulfate is increased by using a product that has a smaller molecular weight. The important point that was not reflected in the Consumer Reports article ("Joint Remedies") is that other factors can be just as important as meeting the label claim of a dietary supplement, especially so in the case of chondroitin sulfate.

The research team at the University of Maryland School of Pharmacy also evaluated the % label claim of chondroitin sulfate vs. price in retail dollars per daily dose. They found that most products costing less than $1.00/dose of 1200 mg of chondroitin sulfate were significantly below label claim. They also found that products costing more than $4.00/daily dose of 1200 mg were clearly below label claims. This showed that there was no clear relationship
between product costs and content. A copy of the University of Maryland study can be found on the ANA website at http://www.amERICANUTRA.com/files/janaeddingtonstudy.pdf.

A problem unique to dietary supplements is that there is no "bioavailability standard" or monograph that dietary supplement manufacturers are required to follow. Good examples of the "bioavailability standard" concept for prescription drugs are digoxin, coumadin, and thyroxine. These generic pharmaceutical products all meet accepted bioavailability standards set by the United States Pharmacopeia (USP). It is important for the consumer to know that quantitative equivalency is not synonymous with either bioequivalency or bioavailability, and no such standards for either currently exist for dietary supplements, or "nutraceuticals."

In our opinion, Consumer Reports inappropriately and incorrectly related truth of label claim and price to bioavailability in its report. By doing this, Consumer Reports may have accomplished exactly the opposite of its intent.

It is clear that when evaluating dietary supplements, each brand should stand on its own original research, and not be assessed by the research of other brands. To compare bioavailability and make an accurate recommendation to buy the least expensive product that is bioavailable when consumed, Consumer Reports would have to test each product for bioavailability. We hope that in future comparisons of dietary supplements containing glucosamine and chondroitin, this fundamental error is not repeated.

We applaud Consumer Reports for attempting to guide the consumer through the maze of dietary supplements now used for joint health. However, when providing consumers with advice on efficacy we refer to the Arthritis Foundation's statement: "When a dietary supplement has been studied with good results, find out which brand was used in the study, and buy that."

In our opinion, until USP or another independent third party develops a "bioavailability standard" or monograph that dietary supplement manufacturers are required to follow for glucosamine/chondroitin products, this is the only safe recommendation to make that incorporates the issues of price, label claim and product content, bioavailability of the product, and efficacy.

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Guest Editorial

Breast Cancer, Hormone Replacement Therapy (HRT) and Diet: Do We Have the Answers Yet?

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In this issue of JANA, Dr. Gina L. Nick has provided a comprehensive review of a key area in cancer/nutrition arena. The breast cancer/hormone replacement therapy controversy is a complex topic, and available data support a cautious approach when a physician discusses HRT with any woman, particularly one with high risk for development of breast cancer. The Nurses Health Study\textsuperscript{1,2} that surveyed over 120,000 registered nurses between 1976 and 1992 clearly demonstrated an overall reduction in mortality among the women receiving HRT (estrogens alone or combined estrogens and progestins). This benefit, due largely to a reduction in death from cardiovascular disease, was diminished substantially by an increase in mortality due to breast and endometrial cancers. Women receiving HRT for a minimum of 5 years had an adjusted relative risk of breast cancer of 1.45. This was particularly true among 60-64-year-old women (relative risk 1.71). Colditz and colleagues concluded that the “tradeoffs between risks and benefits (of HRT) should be carefully assessed.”

Several small studies that Dr. Nick references in her article further demonstrate the correlation between HRT and breast cancer risk. Collectively, these studies support changing a woman’s diet to better manage menopausal symptoms (even in lieu of hormones) and/or to reduce risk of developing cancer. Additionally, a recent published report by American Cancer Society epidemiologists suggests an increased risk of ovarian cancer death with long-term estrogen replacement therapy.\textsuperscript{3}

Breast cancer survivors and women without a breast cancer diagnosis often seek nutritional guidance for many reasons, including weight control, control of menopausal symptoms, and to reduce cancer risk. While controversy exists in many areas regarding the interface of nutrition and cancer, the benefits of a diet rich in whole fruits and vegetables, low in saturated fats, and free of excessive alcohol consumption are well documented. The AICR-World Cancer Research Fund Project summarized the existing scientific evidence for nutritional factors in cancer prevention as either convincing, probable, possible, or insufficient.\textsuperscript{3} They concluded that no one vitamin or mineral supplement (including vitamins E and C, beta carotene, and selenium) has been convincingly shown to be chemoprotective or chemopreventive. While single studies such as that by Favero, as discussed in Dr. Nick’s paper, show an inverse correlation between certain vitamin and mineral supplements and breast cancer risk, these findings have been refuted by other authors.

Not to be forgotten are the importance of weight management and physical activity (as measured by body mass index or BMI), and their benefits to breast cancer risk. Perhaps more controversial, yet no less intriguing are the phytoestrogens and soy components such as isoflavones and their potential inhibitory effect on breast cancer cell growth. These products clearly have estrogenic and antiestrogenic effects. The estrogen receptor/progesterone status, especially among breast cancer survivors, then may be very important when deciding on the use of soy in such a woman’s diet.

Dr. Nick, in Figure 5 at the conclusion of her article, provides excellent suggestions for dietary modification to control cancer risk and menopausal symptoms. This chart will be most useful both to healthcare professionals and their patients.

REFERENCES
Observations of an Emulsified Fish Oil Supplement in a Dialysis Population

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In this issue of JANA, Jones and Kaiser report the results of a pilot study conducted over eight weeks in a dialysis-dependent renal failure population looking at the benefits of supplementation with a novel emulsified fish oil product.

Fish oil supplementation has been studied in a wide array of human diseases. Indeed, if one were to conduct a computer-based MEDLINE search using only the words “fish oil,” it would yield well over 7,000 articles in return. Fish oil has been investigated in the field of nephrology primarily because of its ability to favorably influence lipid levels, to serve as an anticoagulant, an anti-inflammatory agent, and for its anti-fibrotic and anti-sclerotic properties. Anecdotally, investigations of the benefits of fish oil consumption on lipid levels were stimulated by the observation that while inhabitants of the remote Arctic regions were typically overweight and had a high intake of fat in their diets, the incidence of cardiovascular diseases was extremely low. It was noted that the predominant fat in their diet was derived from fish oil, omega-3 polyunsaturated fatty acids, or eicosapentanoic acid. The first reports on the beneficial effects of the use of fish oils in laboratory animals are noted from reports originating in the early 1980s.

Certainly numbered amongst the most famous of the fish oil studies in nephrology is the report published in 1994 by Mayo Nephrology Collaborative Group demonstrating significant reduction in urine protein losses and end-stage renal failure in 106 randomized patients with an inflammatory disease called IgA nephropathy. IgA nephropathy, also known as Berger’s disease, is the commonest primary inflammatory disease of the kidneys, resulting in microscopic hematuria, or blood in the urine. A long-term follow-up of 75 of the patients in the Mayo collaborative study was published in 1999, again demonstrating that early and prolonged use of fish oil supplements delays the progression of kidney disease in patients with IgA nephropathy, and that the benefit of supplementation continued to be evident after many years of follow-up.

Reports of the use of fish oil in a hemodialysis population are far more limited. Fish oil supplements have previously been reported to reduce erythropoietin requirements, and to modify platelet aggregability and lipid levels in a small group of hemodialysis patients. All investigators, however, have not reproduced these findings, and some have found no benefit of fish oil supplementation at all. For that reason, further carefully constructed trials are warranted.

The eight-week pilot study by Jones and Kaiser reported in JANA examines the specific outcomes attributable to fish oil supplementation of reduction in C-reactive protein, albumin levels, hemoglobin levels, and erythropoietin use, a recombinant hormone therapy injected to stimulate new blood cell formation. The rationale for each of these observations is that hemodialysis dependency is known to be a “pro-inflammatory” state. Measurement of C-reactive protein, which is a non-specific marker of inflammation, is known to demonstrate high values in the majority of hemodialysis patients. High C-reactive protein levels are known to correlate with poorer outcomes in a dialysis pop-

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ulation. Indeed, patients with active inflammatory states on dialysis typically have lower hemoglobin levels, lower albumin levels, and higher requirements for erythropoietin at greater expense. Patients with high C-reactive protein levels and low albumin levels are also at increased risk of premature mortality.

The results of this trial are suggestive that C-reactive protein levels might be reduced in a population of hemodialysis patients, and that this reduction in the apparent inflammatory state might also confer the benefits of improved albumin levels and maintenance of satisfactory hemoglobin levels with smaller doses of erythropoietin delivered. Furthermore, subjective observations suggest that these patients might have somewhat increased urine output, reductions in urine protein losses, and improvement in generalized musculoskeletal pain.

The study of Jones and Kaiser is clearly a first step towards designing a placebo-controlled, randomized trial to determine whether fish oil supplementation will prove to be both cost effective and beneficial to hemodialysis patients.

The preliminary results of the pilot study are certainly encouraging in that regard.

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**CONGRESSIONAL UPDATE**

**Congress Acts on Dietary Supplement Issues**

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Since the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994, the U.S. Food and Drug Administration (FDA) and Congress have been reluctant or unable to designate financial resources necessary to implement and enforce the law. This became even more apparent after September 11 when FDA and Congress turned their attention toward bioterrorism and food safety. Despite a host of competing priorities during the last federal appropriations cycle, Congress was finally able to designate several million dollars specifically for dietary supplement programs.

In 2002, the Congress will also continue consideration of legislation that will allow food stamps to be used to purchase vitamins, minerals, and other products to enhance nutrition for qualified recipients. The Congress is also expected to resolve differences between House and Senate bills that would require registration of all facilities that manufacture, handle, pack, or process food, including dietary supplements. This article is a summary of recent Congressional Action.

**FEDERAL FUNDING FOR DIETARY SUPPLEMENT REGULATORY SYSTEM AND SCIENTIFIC RESEARCH**

Because of several competing priorities at FDA, dietary supplements have traditionally not received adequate agency attention when compared to other food products and food ingredients. In an effort to address the situation, President Bush has signed into law an appropriations bill for...
FDA that includes $2.5 million for the agency to update and modernize the adverse event reporting system for dietary supplements. A Congressional committee held hearings in 1999 that were quite critical of the agency’s ability to collect, analyze, and maintain adequate information on the safety of particular dietary supplement products and there appeared to be little or no follow up with manufacturers or healthcare officials. The designation of $2.5 million will allow FDA to develop specific procedures for dietary supplement adverse event reports and have those reports evaluated by scientific personnel that are adequately trained to communicate their findings for appropriate follow-up.

The Congress has also approved $500,000 for FDA to enhance enforcement activities relating to unlawful labeling of dietary supplements and an additional $500,000 to augment the resources of the Office of General Counsel of the Department of Health & Human Services (HHS) for legal activities. The federal appropriations bills signed by President Bush also contain language from House and Senate conferees to strongly urge HHS Secretary Tommy Thompson to publish proposed good manufacturing practices for dietary supplements. GMPs were authorized by DSHEA in 1994, but FDA has failed to publish a GMP proposal.

In addition to safety and enforcement, the Congress has approved additional money for the National Institutes of Health (NIH) and FDA to develop the science regarding dietary supplements. The Office of Dietary Supplements at NIH will receive $17 million in the next fiscal year that will allow the office to speed up collaborative efforts to develop, validate, and disseminate analytical methods and reference materials for the most commonly used botanicals. The National Center for Complimentary and Alternative Medicine (NCCAM) will also receive $104.6 million, a portion of which will be used in conjunction with other institutes to appropriately study the science of certain dietary supplement ingredients. An additional $1 million has been designated to continue the work of FDA, in collaboration with the National Center for Nutritional Products Research at the University of Mississippi, to identify and analyze botanical ingredients.

Unquestionably, Congress, FDA, and NIH made great strides in the last federal budget cycle to more appropriately regulate and study dietary supplement products, including vitamins, minerals and other botanical ingredients. In 2002, the Federal Trade Commission (FTC) will also continue its mission by initiating enforcement actions against false and deceptive advertising claims.

SENATE COMMITTEE APPROVES SUPPLEMENT PURCHASES WITH FOOD STAMPS

The U.S. Senate Agriculture Committee has approved legislation that will allow qualified families to use food stamps to purchase nutritional supplements. As part of a massive reauthorization of federal farm, food, and nutrition programs currently under debate on the Senate floor, the provision is intended to encourage more low-income families to purchase vitamins and minerals, such as folic acid, iron, and calcium. Proponents of the measure argue that allowing food stamp recipients to purchase nutritional products will have positive healthcare outcomes on a segment of the population where improvements in nutrition are needed the most.

BIOTERRORISM INCLUDES REGISTRATION FOR FOOD MANUFACTURERS

The U.S. House and Senate have passed competing versions of legislation to improve the national preparedness for a bioterrorist attack, including a requirement for food manufacturers and processors to register all facilities with FDA. The two pieces of legislation authorize the spending of federal funds for bioterrorism preparedness to protect food supplies, improve the Centers for Disease Control and Prevention’s medical response, and allow for HHS to expand the national stockpile of antibiotics and vaccines. The facility registration provisions in both bills apply to manufacturers of dietary supplements as well as conventional foods. Both bills also allow for the administrative detention of imported foods that pose a potential threat of adverse health consequences, maintenance and inspection of records for foods, and prior notice of imported food shipments.

A conference committee will be required to resolve the differences between House and Senate versions of the bioterrorism bills. President Bush is expected to sign the conference report into law.

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Biological and Chemical Terrorism: Natural Cures or Bogus Claims?

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The events of September 11, 2001 have changed the realities of life in the United States of America. We are faced with a new sense of vulnerability that has not existed in the US since the attack on Pearl Harbor.

Among local, state and governmental agencies, the Public Health system was challenged to provide an infrastructure to effectively respond to the challenges of disasters and emergencies that can threaten the integrity of our national health. The potential for further attacks using nuclear, biological or chemical agents has focused the attention of the US public and healthcare community at large on the issue of diagnosis and management of diseases resulting from such agents.

Documented cases of anthrax infections (cutaneous and inhalation) have occurred since October 4 throughout five US states, claiming the lives of five citizens and exposing thousands to the disease. Many public health officials openly discussed the possibility of the intentional use of lethal agents such as smallpox and plague, and the protective measures to be taken in event of their occurrence.

Concerned citizens called their doctors’ offices and the local public health departments demanding preventive prophylaxis with antibiotics and vaccinations. Since October 8, approximately 32,000 persons with potential exposure to B. anthracis in four states and the District of Columbia have initiated antimicrobial prophylaxis to prevent anthrax infection, and for approximately 5,000 persons, a 60-day course of antibiotics was recommended. Unfortunately, few data exist regarding the use of these antimicrobials for longer periods, and recent Centers for Disease Control (CDC) recommendations suggest extending the post-exposure prophylaxis to 90 days.

Between October 26 and November 6, 2001, an epidemiologic evaluation to detect adverse events associated with antimicrobial prophylaxis was conducted among 8,424 postal employees who had been offered such prophylaxis for 60 days in New Jersey, New York City, and one postal facility in the District of Columbia. During this period of time 8,424 postal employees were offered antimicrobial prophylaxis, 5,819 (69%) completed or were administered a questionnaire to evaluate the occurrence of adverse events.

A total of 3,863 (66%) had initiated antimicrobial prophylaxis; of these, 3,428 (89%) reported using ciprofloxacin and 435 (11%) used other antimicrobials (when ciprofloxacin was contraindicated), including doxycycline (6%) and amoxicillin (1%). Of the 3,428 persons on ciprofloxacin, 666 (19%) reported severe nausea, vomiting, diarrhea, or abdominal pain; 484 (14%) reported fainting, light-headedness, or dizziness; 250 (7%) reported heartburn or acid reflux; and 216 (6%) reported rashes, hives, or itchy skin. Of those persons taking ciprofloxacin, 287 (8%) discontinued the medication; 116 (3%) discontinued the medication because of adverse events, 27 (1%) discontinued because of fear of possible adverse events, and 28 (1%) stopped taking the drug because they "did not think it was needed". The survey did not indicate if these patient
received probiotic therapy to support their gastrointestinal physiological function.

Probiotics are defined as the microbial food supplements that beneficially affect the host by improving its intestinal microbial balance. Probiotics are the health enhancing functional food ingredients used therapeutically to prevent diarrhea, improve lactose tolerance and modulate immunity. Lactobacillus, Bifidobacterium, and several other microbial species are used as probiotic components to exert such effects by restoring the composition of the gut microbiota affected by antibiotic-therapy. The beneficial role of such nonpathogenic bacteria in the management of antibiotic induced intestinal dysbiosis should be the subject of randomized-controlled trials to assess the clinical application of such modalities. The safety and specification of a particular probiotic agent and methods of delivery to a particular population for a specific purpose should be carefully documented before making clinical recommendations. Physicians who put patients on long-term antibiotic therapy should consider a recommendation for probiotic supplementation while the patient is on such therapy.

Unfortunately, the fear of potential infection by bioterror agents and the concern of many Americans about potential adverse effects of antibiotics therapy were abused by opportunistic business people. Hundreds of websites have mushroomed selling everything from mail sterilizers to oregano oil to prevent and treat illnesses such as anthrax. A BBC news report discovered more than 200 sites marketing bioterrorism-related products including gas masks, protective suits, mail sterilizers, biohazard test kits, homeopathic remedies and dietary supplements. The sites are claiming that dietary supplements such as colloidal silver, zinc mineral water, thyme and oregano oil are effective treatments for illnesses such as anthrax.

On other web sites herbal remedies such as echinacea, garlic and Dr. Christopher’s "Anti-Plague" formula are being featured as "treatment" options to cure Anthrax.

The mayor of a tiny central Florida town suggested colloidal silver, an elixir of metallic silver particles in demineralized water as a "cure" for anthrax. He planned to buy a colloidal silver generator for the town of 956 residents, located 30 miles northwest of Orlando and encouraged town residents to drink the potion as a "simple solution" to biological threats such as anthrax.

The mayor was forced to step down two months after proclaiming that a silver-laced liquid could cure anthrax and other ills, saying that he was the victim of a "Jihad" against him.

The FTC, with the help of the Food and Drug Administration (FDA), more than 30 state attorney generals, and the California Department of Health Services, have cracked down on such quackery, focusing on web sites offering products claiming to protect against, detect, prevent, or treat biological and chemical agents, including anthrax. More than 200 sites marketing bioterrorism-related products were uncovered, and additional sites are being evaluated for possible warning letters. Included in the review were such items as gas masks and protective suits; mail sterilizers; biohazard test kits, homeopathic remedies, and dietary supplements such as colloidal silver, zinc mineral water, thyme, and oregano oil as treatments for contamination by biological agents.

Web sites may be subject to state or federal investigation or prosecution for making deceptive or misleading marketing claims that their products can protect against, detect, prevent, or treat biological or chemical contamination. "This marketing targets people worried about the prospect of exposure to lethal biological or chemical weapons. The FTC is aware of no scientific basis for any of the self-treatment alternatives being marketed on the Internet," said Howard Beales, FTC's Director of Consumer Protection. "Essentially, these operators need to shut down these areas of their sites or face prosecution".

On November 5, 2001, dietary supplement trade associations (American Herbal Products Association, Consumer Healthcare Products Association, the Council for Responsible Nutrition, the National Nutritional Foods Association and the Utah Natural Products Alliance) issued a press release emphasizing that dietary supplements have not been proven to treat or prevent anthrax and that therapies for the treatment or prevention of anthrax should be recommended only by qualified public health authorities.

The American Nutraceutical Association was established in 1997 to develop and provide educational materials and continuing education programs for health care professionals and consumers on nutraceutical technology and science. We strongly support federal regulations that do not allow dietary supplements to claim to treat or prevent any disease, including anthrax.

We will continue to promote the rational application of dietary and nutritional supplements such as probiotics that may be used concurrently with ciprofloxacin for antimicrobial prophylaxis, and support clinical studies to assess the benefits of such modalities as an integral part of current disease management protocols. However, we encourage our members and readers to criticize and reject the natural "bioterror cures" and to call those by their name: sham and quackery.
Mark Houston, MD, SCH, FACP, FAHA
Editor-in-Chief of JANA

In October 2001, Dr. Houston accepted the appointment of Editor-in-Chief of JANA. The following interview was conducted by Allen Montgomery, RPh, ANA CEO and Executive Director.

Montgomery:
Dr. Houston, I am pleased to introduce you to our readers as the new editor-in-chief of JANA. Can you share with JANA readers some details about your medical background, both as a practitioner and an educator?

Houston:
I graduated from Vanderbilt University Medical School, and completed my internship in internal medicine at the University of California, San Francisco. For 12 years, I was a full-time faculty member at Vanderbilt University School of Medicine, serving as an Associate Professor of Medicine, Co-Director of Medical ICU, Chief of Clinical Section-General Internal Medicine, and as Medical Director of the Executive Physical Program. I am still a Clinical Professor of Medicine at Vanderbilt, but have left the full-time faculty.

In 1990 I moved my practice to Saint Thomas Hospital. There, I founded the Hypertension and Vascular Biology Institute, along with the Life Extension Institute, and serve as medical director. I have published over 110 medical articles in peer-reviewed journals and abstracts; written over 20 books, book chapters and monographs; penned over 50 newspaper and magazine articles; and serve as either a member of the editorial board or editorial consultant for 20 medical journals including the New England Journal of Medicine (NEJM), and the Journal of the American Medical Association (JAMA).

Montgomery:
What do you see as the future role of JANA?

Dr. Houston:
My goal is to make JANA the premiere clinical and nutraceutical journal in the USA – with an emphasis on clinical practice. I want JANA to provide guidance to MDs, pharmacists, osteopathic, chiropractic and naturopathic physicians, nutritionists, and dieticians, as well as other members of the healthcare delivery system, on the current research in nutrition and nutraceuticals for the prevention and treatment of disease. Editorially, we will cover specific topics and new research pertinent to virtually all organ sys-

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tems of the body, with emphasis on neurological disease, cardiovascular disease, immune function, gastrointestinal disease, arthritis, diabetes, and cancer. This focus on the use of nutraceuticals in clinical practice is vital, for it has been proven that about 50% of all diseases affecting the U.S. population are nutritionally related. Up to 70% of those diseases could either be prevented, or their severity reduced, by incorporating proper nutrition and the appropriate nutraceuticals into a daily healthcare regimen. Our mission at JANA is to educate and guide healthcare professionals on these topics.

The strength of any journal is dependent on the editorial board and the scientific quality of the articles submitted. I have expanded the editorial board and have added leading MDs, PharmDs, PhDs, NDs, and others that are affiliated with institutions such as the University of Maryland School of Medicine, Vanderbilt University School of Medicine, the University of Tennessee School of Medicine, the Medical University of South Carolina, the Department of Optometry at the VA Medical Center in Chicago, and the Department of Health and Exercise Science at Colorado State University. These professionals will serve as consulting editors and members of the editorial board. We will continue to seek qualified members who share our goals and wish to become affiliated with JANA. Anyone interested in serving on our editorial board should submit their curriculum vitae and formal request to me at mhoustan@ana-jana.org.

From an editorial point of view, we will continue to seek submissions that are based in good science, and that provide useful data applicable to the clinical practice. I encourage researchers, companies and others involved in clinical studies to submit manuscripts for consideration to:

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Instructions for authors can be found on our website at www.ana-jana.org. Click on JANA, then scroll to the bottom of the screen for “instructions for authors.” Authors with additional questions can direct them to our editorial office at 205-980-5710.

Montgomery: You are recognized nationally as an expert in the diagnosis and treatment of hypertension. What experiences have you had in the use of nutrition and nutraceuticals in its prevention and treatment, and how have your patients reacted to these approaches?

Dr. Houston: Hypertension affects about 40% of the adult U.S. population. It is a genetic disease with strong environmental influences. One factor is nutrition. About half of the mild or stage I hypertensive patients could have their blood pressures controlled to normal levels utilizing a scientifically based nutrition and nutraceutical program. I have seen this happen in my practice in the last 10 years.

I’ve spent the last twelve months reviewing the world’s scientific, English-language medical literature on the role of nutrition and nutraceuticals in the prevention and treatment of hypertension. From this literature search, I have completed a 75-page manuscript with over 700 references on the topic “The Role of Vascular Biology, Nutrition, and Nutraceuticals in the Prevention and Treatment of Hypertension.” The manuscript documents the efficacy of such a program. This manuscript is undergoing peer-review and will be published soon in JANA. I am also exploring with major publishers the potential of developing a book on this topic for both consumers and healthcare professionals.

As for patient response, the vast majority of my patients prefer and seek non-pharmacologic management of their hypertension. This includes nutrition, nutraceuticals, vitamins, antioxidants, exercise, weight reduction, and other modalities. I’ve been delighted to see blood pressure return to normal levels in about 80% of patients who follow the treatment regimen I have directed. Side effects with this nutritional and nutraceutical approach are basically nonexistent. Instead of side effects, patients sense an increase in their well being, energy, and mental focus. I find that in most cases their overall quality of life improves.

Montgomery: Have your colleagues adopted or become more accepting of the use of nutraceuticals in a traditional medical practice?

Dr. Houston: There is an increasing interest among traditional physicians in learning and applying non-traditional complementary and integrative approaches. The point I want to make is that physicians must be provided with scientific validation of any tool they may use in their practice. This is why it’s so important for nutraceutical companies to develop strong research programs. As physicians, we cannot recommend products or services that are not supported by good science and clinical studies. As more and more nutraceutical products are validated by clinical studies, more traditional physicians will use them in their practices. I personally use a clinically validated nutraceutical product, (glucosamine/chondroitin) for the management of osteoarthri-
tis. I only use this and other nutraceutical products that are supported by well-controlled clinical studies.

It is important for physicians to become better educated in nutrition and the use of nutraceuticals. The majority of consumers that I meet at various speaking engagements throughout the United States are seeking physicians who are open to and knowledgeable about integrative medicine. Data shows that the per capita expenditure on complimentary medicine is increasing over traditional medicine. Over time, more and more consumers will seek out physicians who are knowledgeable in these topics and can provide guidance on them.

Montgomery:

Your medical training and practice would be considered traditional. What led you into complementary and integrative medicine, and how have your patients responded to these approaches?

Dr. Houston:

It began as a personal issue related to my own health, and that of my father who had prostate cancer. I learned as much as I could about preventing and treating prostate cancer with nutrition and nutraceuticals. I used this in conjunction with traditional medical treatments.

As I learned more about the role of nutrition and cancer, it became more apparent how they are connected. Nutrition also has a tremendous impact on cardiovascular disease, neurodegenerative diseases, aging, diabetes, and virtually all other diseases.

Unfortunately, traditional MD training pays very little attention to the use of nutrition and nutraceuticals. Thus, like many other physicians, I continue to educate myself independently of my formal medical training. In fact, I am currently working on a two-year MS degree in nutrition.

In my opinion, successful physicians of the future will be those who can guide their patients in traditional medical treatments as well as complementary, or integrative medicine, with an emphasis on nutrition and the proper use of clinically validated nutraceuticals, vitamins, minerals and other dietary supplements.

To point out how quickly times change, when the American Nutraceutical Association was founded in 1997, the word nutraceutical was not even in the dictionary. Today, it is defined by Webster’s as nutraceutical n. A food or naturally occurring food supplement thought to have a beneficial effect on human health. It is important that physicians, pharmacists, nurses, nutritionists, dieticians, chiropractic, osteopathic and naturopathic physicians learn what is happening in the field of nutraceuticals and complementary medicine. I want to make JANA one of the primary sources to help guide and educate these healthcare professionals on these topics.
Why Funding for Nutraceutical Clinical Trial Research Will Remain Minimal

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INTRODUCTION

Question: If selected dietary supplements, such as vitamins, minerals, herbals, food substances or their combinations, are beneficial for specific chronic conditions or illnesses, or to maintain wellness, should we expect major pharmaceutical drug companies and/or government agencies to fund nutraceutical human clinical trial research in order to more precisely assess and validate such benefits? That is, if there were anything worthwhile to pursue, surely the major pharmaceutical drug companies and/or the government would be among the first to seize the opportunity for improvements in health care. Given governmental medical panels’ recommendations of many key vitamins and minerals, there is already orthodox acceptance of some of these products’ beneficial health value. Moreover, financial disclosures from pharmaceutical companies note that many are losing some of their high-profit-profile patents and are anxiously seeking new avenues for drug successes. If some elements of the over-the-counter dietary supplement market have usefulness, particularly for preventative care and for maintenance of wellness, as well as for specific chronic diseases and chronic conditions, should we not expect pharmaceutical companies as proven, efficient developers of drug remedies to seize the opportunity to demonstrate their value through clinical trial research? Moreover, when a scientific case can be made for potential benefits of some nutraceuticals, do not appropriate government research-directed agencies have responsibility to serve the public through funding of human clinical trials, furthering the prospects of low-cost, effective interventions and/or preventative measures? In this commentary, we focus on why major pharmaceutical companies have not pursued dietary supplement clinical trials research in primary or secondary prevention and why we should also expect government funding to continue to be modest in such areas.

PHARMACEUTICAL COMPANY DISINCENTIVES

The lack of incentives for conducting clinical trials on nutraceuticals on the part of pharmaceutical companies (commonly called “Big Pharma” in the industry) can be clearly delineated. The first is that it is quite unlikely that Big Pharma could make significant profit, or possibly any profit at all, from such a venture. In conducting the trial, sponsors must compensate collaborating principal investigators for their administrative roles. Additional costs include trial participant solicitation, participant monitoring charges such as lab and radiologic studies, data recording and analysis, and administrative overhead. Separately added in is the cost of products to be tested. These expenses recur over several years. To measure therapeutic impact, trials are typically targeted for at least five years, with recruitment projected to take several years.

Dr. Joseph DiMasi and his research team at Tufts University have been issuing research cost estimates for years based upon proprietary surveys of top drug companies.1 In December, 2001, new cost estimates were released; preclinical out-of-pocket expenses averaged $121 million, while clinical costs averaged $282 million.2 Presumably these clinical costs include actually bringing the drug to mar-
Neither the products of the marketplace, nor those of the government, are likely to become a standard of care, diminishing expectations of the large and consistent sales Big Pharma need to enter the marketplace.

In summary, Big Pharma would be correct to summarize that there is little, if any, money to be made in this area, that any advantage of nutraceutical research trials would be short-lived, and that the prospects of losing substantial investment are great. Thus one should hardly fault Big Pharma for avoiding this area. If some top-level managers of a Big Pharma firm were to decide to embrace such a mission, we could readily expect its shareholders to elect top-level managers who understood what their mission really is.

GOVERNMENTAL/ACADEMIC DISINCENTIVES

Governmental disincentives differ substantially from those affecting Big Pharma. Governmental agencies funding research and clinical trials emanate primarily from various divisions of NIH, so we will refer to NIH as an eponym for its individual institutes and programs. Some analysts are encouraged by the observation that NIH is funding, for example, a 3000-patient study on Ginkgo for just $15 million, but unfortunately the 3000 patients may yield data which would most likely support a call for a much larger definitive trial, rather than being able to yield unequivocally definitive patient recommendations. It is informative to look at the National Cancer Institute’s (NCI) SELECT trial which explicitly attempts to determine policy recommendations concerning whether selenium and/or vitamin E have a protective role in cancer prevention. We could assume that its extremely highly visible stature and surrounding publicity compelled NCI to carefully craft its patient accrual size and scrutinize the trial’s cost. The trial’s projected cost is $180 million, with a 7 year accrual period to recruit approximately 32,000 people for its various treatment arms for at least a five year testing period. Although merely one example, NCI’s goal of reconciling public policy in this area suggests that these costs and patient numbers support the case for the non-trivial price for such undertakings. Even with governmental support, the profit potential for commercial firms supplying nutraceuticals is extremely modest. For example, if the vitamin E arm of the SELECT trial proved efficacious, one can obtain an entire year’s supply of vitamin E for $15.32 from a common, popular mail order catalogue.3 If the seller even nets 25% of the price before taxes, the less than $4 net per year per patient speaks for itself in terms of investment attractiveness.

In defense of the NIH, one can point to the establishment of the National Center for Complementary and Alternative Medicine (NCCAM) as an indication of expanded government interest in areas including nutraceuticals. This demonstrates that the government is not without some worthwhile initiatives, but useful perspective is gained by noting the NCCAM’s allocated fiscal year (FY)
2001 budget is $89 million against the backdrop of an approximately $20 billion NIH budget, or less than half a percent in support of cancer research studies. NCCAM reports that there was a three-fold increase spent in FY 2000 over FY 1999. As favorable as this may first appear, the actual dollar value spent in FY 2000 was $4 million versus less than $1.5 million the preceding year specifically for cancer research. In contrast, in FY 1999, the National Cancer Institute estimates it spent $3.2 billion on cancer research. Given that NCCAM came into existence in FY 1999, one can point to exponentially increasing budgets over recent history. With prior research funding at much lower levels, the situation has certainly improved. Nonetheless, in absolute dollars, given the fiscal challenge of this area of research, the funding prospects remain sobering. Thus it is reasonable to inquire as to the underpinnings of such relative reservations regarding nutraceutical research, for indeed, the problems are challenging.

First, the area of nutraceutical marketing historically has been rampant with ill-conceived claims and false promises and has been tainted with quackery and hyperbole. Perceived legitimate medical science after World War II has focused on disease per se and its treatment; prevention as a whole has been quite the step-child, if not the orphan. Funding sources can respond only to those seeking funding, and new researchers could hardly be faulted for avoiding a field that in its past was rife with charlatans and quackery. Peer-review boards allocating funds could hardly be faulted for funding treatment research over more historically dubious endeavors in disease prevention or health enhancement.

Another factor contributing to the disinterest in nutraceutical research has been the myth that the American diet was either adequate or could be; from this viewpoint, research on the impact of nutraceuticals’ needlessly wastes precious dollars. Even as the integrity of the American diet declines precipitously, this myth prevails, even while more and more data evolve questioning its premises. A significant portion of the scientific community appear to embrace a traditionalist American ethic that says we can address the issue of nutrients if we would just eat from the food pyramid which the government has insured is displayed in every elementary school classroom around the country. With this cultural perspective, funding research in the area of disease prevention or health maintenance through the peer-review process could hardly be expected to yield a great deal of enthusiasm. This cycle of indifference has historically fed upon itself, with the lack of previously funded research supporting the proposition that there was little support for further research.

FUTURE FUNDING EXPECTATIONS

In light of these barriers, it is remarkable that there has been as much research to date as has been published for nutraceuticals. Much of what transpires is often short-term, highly specific-agent focused, and more often than not, mouse-model-directed with regard to acute disease states. This permits researchers to keep expenditures low, adhere to models of single-agent focus, for a specific disease, and still have publishable results. The counter-point of course is that such research has less salience for humans over long intervals or with chronic conditions. Consequently, skeptics may demand far more conclusive evidence before any public policy recommendations may be derived. As a consequence, the research fails to lead to definitive conclusions, only reinforcing the recognition that funding should be directed to more productive arenas.

One response to this funding conundrum is an alternative funding model proposed by some investigators, involving fee-for-service research. In this model, healthy individuals or suitably non-acute-sick patients who can give informed consent are asked to pay a modest fixed fee toward the cost of such clinical trial research. This model incorporates the interested public into the funding equation as an informed and consenting funding participator. Another recently evolved model has been successful business people (e.g., “dot-com” multimillionaires) who fund selected projects that interest them personally. These alternative models are relatively embryonic. For the foreseeable future, we should expect virtually no funding by Big Pharma and relatively modest levels of funding from the government in the nutraceutical clinical trial research area, which nonetheless offers potentially significant, beneficial outcomes, typically at low product cost and often with few side effects.

REFERENCES

2. Ibid.
New Developments in the Prevention and Treatment of Neurodegenerative Diseases Using Nutraceuticals and Metabolic Stimulants

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INTRODUCTION

Neurodegeneration was once considered to be a simple acceleration of the normal aging process. Aging of the brain, however, generally produces little deterioration of neurological function. Neurodegeneration on the other hand, results in an appreciable loss of cognitive and motor function. Hence, a significant loss of cognitive ability is always pathological. In this review I will discuss several changes that occur with neurodegeneration and offer potential ways to reduce one’s risk of developing a neurodegenerative disorder.

THE CENTRAL MECHANISM OF NEURODEGENERATION

When one reviews the extensive literature on neurodegeneration, one finds many seemingly unrelated pathological events, such as excitotoxicity, viral inflammation, autoimmune reactions, trauma, cerebrovascular impairment, and metal toxicity. Surprisingly, a single central mechanism explains all. This mechanism is a combination of excitotoxic injury coupled with free radical damage to neural tissue. Excitotoxins are neurotransmitters, such as glutamate or aspartate, that can cause cell death when their actions are prolonged. These chemicals are thought to play and important role in ischemic brain damage.

A free radical molecule has an unpaired electron in its outer orbital, one that robs surrounding molecules of their electrons, generating a process referred to as oxidation or oxidative stress. The particles responsible for this oxidative injury are referred to as reactive oxygen species (ROS). A related particle, discussed less often in the lay literature, is the reactive nitrogen species (RNS). Its nitrogen atom interacts chiefly with amino acids, such as tyrosine, interfering with numerous biochemical processes in the central nervous system. When these particles react with tyrosine they form nitrotyrosine, a measurable marker for RNS damage. As we shall see, these oxygen and nitrogen products are commonly found in the tissues of those with neurodegenerative disorders, such as Alzheimer’s dementia, Parkinson’s disease, Huntington’s disease and Lou Gehrig’s disease (ALS).

The excitotoxic process entails a complicated series of reactions involving the release of the amino acid neurotransmitter glutamate. Glutamate acts at a series of receptors on the neuron’s surface that in turn, either directly or indirectly, control the calcium pore or channel. This channel tightly regulates the entry of calcium into the neuron. Calcium homeostasis is critical because its loss is the trigger for numerous abnormal signaling systems in the neuron, which when over-stimulated can precipitate the destructive generation of free radicals and inflammatory reactions that can ultimately lead to the death of the cell.

For this reason, glutamate levels outside the neuron are

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carefully regulated. Even small elevations in glutamate can precipitate the destructive reactions we refer to as excitotoxicity. Glutamate content outside the neuron is controlled by a re-uptake system that involves a series of glutamate transport proteins.\(^1\) Should too much calcium enter the neuron, other cellular mechanisms act to remove it, either by moving it into the mitochondria, pumping it outside the neuron, or sequestering it in the endoplasmic reticulum.\(^4\) All of these processes require cellular energy. When cellular energy supplies fall, these protective systems fail.

Calcium acts as a biochemical trigger for numerous reactions, all of which play a vital role in neuron function, such as nitric oxide signaling information, activation of special eicosanoids and regulation of the neuron’s gene messages.\(^5\) When too much calcium enters the cell, it triggers an excessive production of nitric oxide, a cell-signaling molecule.\(^6\) As the nitric oxide begins to build up, it interacts with the superoxide radical to produce the highly reactive and destructive peroxynitrite radical. This radical wrecks havoc on the mitochondria, producing injury to its enzymes (electron transport chain) and in addition, damages mitochondrial DNA.\(^7\) A significant loss of cellular energy production results.

Excess calcium also stimulates the activation of the enzyme protein kinase C, which activates the membrane-bound enzyme, phospholipase A\(_2\) (PLA\(_2\)).\(^8\) This enzyme in turn releases arachidonic acid from the membrane lipid stores, where it is then acted upon by two enzymes, cyclooxygenase (COX) and lipoxygenase (LOX), which convert it into numerous reactive molecules called prostaglandins and leukotrienes. Both metabolic products, when present in excess, can drastically increase free radical production.\(^9\)

As the level of free radicals begin to rise, they interact with the lipids in the cell’s various membranes, setting up a chain reaction called lipid peroxidation. The peroxyl radical plays a major role in membrane injury as well as injury to mitochondria.\(^10\) As the destructive process spreads through the membrane, secondary metabolic products are produced, such as 4-hydroxynonenal, which can be even more destructive.\(^11\)

Cellular proteins are building blocks for the hundreds of enzymes used by each cell to function. Free radicals interact with both proteins and carbohydrates in the cell, causing conformational changes in their structure. While free-radical-altered proteins, called carbonyl products, increase with aging, they don’t increase to the extent we see in the tissues of those with neurodegenerative diseases.\(^12,13\)

Another cell component damaged by free radicals is DNA. The cell contains two sets of DNA: one type in the nucleus, and another in each of the cell’s numerous mitochondria. Mitochondrial DNA is especially vulnerable to oxidation reactions, being about 10X more sensitive to free radical damage.\(^14\) This is important because with aging, oxidized DNA begins to accumulate, resulting in dwindling cell energy supplies.\(^15\) Cellular low energy causes reduced function of the neurons and increased sensitivity to excitotoxicity. The susceptibility of mitochondrial DNA to free radical oxidation increases with age, being 15X more active after age 70. As this process accelerates, a special autodestructive gene, called the p53 gene, is activated.\(^16\) Its purpose is to kill the neuron when the cell is so badly damaged that it cannot be restored to health by the cell’s reparative enzymes.

Much of the destructive change seen in neurodegenerative disorders, at least in the earlier stages, does not entail neuron death. Several studies have shown that in the case of Alzheimer’s disease, most of the damage is directed at the neuron processes, such as the dendrites and synapses.\(^17\) While we do not completely understand the role played by \(\beta\)-amyloid peptides, we do know that much of their destructive potential comes from the free-radical-generating molecule hydrogen peroxide.\(^18\) Amyloid has also been shown to enhance excitotoxicity.\(^19\)

Another pathological characteristic of Alzheimer’s disease is the presence of microscopic neurofibrillary tangles composed of over-phosphorylated tau protein. Recent evidence has demonstrated that the lipid peroxidation product 4-hydroxynonenal interacts with the tau protein to accelerate this process, and prevents the tau proteins from dephosphorylating.\(^20\) Several of the transition metals such as aluminum and mercury, and exposure to MSG can precipitate the same event experimentally.\(^21\)

Finally, the entire process involves an overreacting immune system apparently triggered by excitotoxicity and free radical injury.\(^22\) The microglial cell, the cellular basis of central nervous system immunity, is activated by any event that increases the free radical-excitotoxicity cascade.\(^23\) As we shall see, CNS immune activation plays a major role in neurodegeneration.

This entire process appears to be the same for numerous conditions including autoimmune diseases, viral encephalitis, Lyme disease, AIDS dementia syndrome, brain injury, strokes, heavy metal toxicity, spongiform encephalitis (Mad Cow disease), and most of the degenerative brain disorders, such as Alzheimer’s dementia, Parkinson’s disease, Huntington’s disease, and ALS.

**IRON AND NEURODEGENERATION**

It is known that as we age our brain accumulates more free iron.\(^24\) In the past it was assumed that only free iron was harmful; recent evidence indicates that even iron combined to ferritin can damage neurons.\(^25\) Excessive iron accumulation is seen in many neurodegenerative disorders, including Alzheimer’s dementia, Parkinson’s disease, and ALS. In biological systems, iron is known as one of the most powerful free radical generators.
Recent evidence indicates that those at risk of developing Parkinson’s disease have a defect in iron metabolism. In this study, Parkinson patients’ total iron binding capacity and transferritin saturation were significantly lower than that of controls, with no difference in their dietary intake of iron. Other researchers have found increased iron and aluminum in the neuremelanin pigment in the substantia nigra of Parkinson’s patients. Aluminum appears to displace iron from the ferritin molecule, thereby increasing the interaction of iron during the hydrogen peroxide interaction with superoxide. This reaction forms the powerful hydroxyl radical.

In the brains of Alzheimer’s disease patients, aluminum, iron, and mercury are consistently found in elevated concentrations in affected neurons, transferrin levels are decreased, and iron, ferritin, and transferrin are concentrated around the senile plaques.

**ALUMINUM AND NEURODEGENERATION**

The connection between aluminum exposure and brain dysfunction was strengthened when several dialysis units reported patients with an unusual dementing syndrome related to elevated aluminum levels in the dialysate. Once the dialysis water was cleared of aluminum, the dementing syndrome disappeared. Based on this finding, others began to suspect aluminum toxicity as an etiology of Alzheimer’s disease.

One early study, in which individuals were examined in 88 counties in England and Wales, areas with elevated aluminum levels in the drinking water had higher incidences of Alzheimer’s dementia. A later, more well-controlled study found that elderly people who drank water high in aluminum had a 4.4X higher incidence of Alzheimer’s disease than those who drank water with lower levels.

After this suggestive research, more accurate studies were conducted for measuring brain levels of aluminum in several neurodegenerative disorders. Despite early conflicting results, the latest studies performed with microtechnique high-tech laser and x-ray probes clearly indicate elevated levels of aluminum in the area of neurofibrillary tangles in Alzheimer’s disease. Similar results have been found in cases of Parkinson’s disease. The results of one ALS study indicated that while spinal cord levels of aluminum were not elevated above controls, they did find a 1.5- to 2-fold elevation in iron and calcium. Using more sophisticated methods, another study confirmed the earlier finding of elevated aluminum levels in the motor neurons of ALS patients.

Besides increasing free radical generation, aluminum has several other negative effects on cell function. One study found that primates exposed to excess aluminum had a significant decrease in total lipid, glycolipid, and phospholipid content in their brains. Aluminum also damages membrane-bound enzymes such as Na+-K+-ATPase, acetylcholinesterase, and 2',3'-cyclic nucleotide phosphohydrolase, all enzymes necessary for normal neuron function.

A recent study found that aluminum in the presence of melanin significantly enhanced lipid peroxidation. This is important in the case of Parkinson’s disease, since the neuremelanin-containing cells of the substantia nigra are the cells most affected by the disease. Of enormous importance is the finding that high aluminum levels can inhibit the activity of many antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase.

Several studies have linked aluminum to formation of the paired helical filaments found in Alzheimer’s disease. Aluminum appears to interfere with dephosphorylation of the hyperphosphorylated tau protein. Experimentally, using the aluminum-chelating agent desferrioxamine, researchers could reverse this resistance to aluminum-induced dephosphorylation.

The entry of aluminum into the brain, past the blood-brain barrier, is significantly enhanced when aluminum is bound to glutamate. Once in the brain, aluminum has been shown to potentate excitotoxicity by enhancing glutamate-triggered calcium accumulation within the neuron, and to increase the formation of iron-induced free radicals.

Is aluminum the only cause of these neurodegenerative diseases? I don’t think so. However, I do think that it is a significant contributing factor. Numerous environmental agents, viruses, autoimmune disorders, and injuries can all trigger the same central destructive mechanism—excitotoxicity. Often we see several of these factors coexisting in the same person. At high risk is the person having mineral deficiencies, poor nutritional supply of antioxidants, and antioxidant enzyme deficiencies.

It is interesting to note that gastrointestinal absorption of aluminum was found to be enhanced in Down’s syndrome, a condition with pathological features similar to Alzheimer’s disease. In this study, aluminum absorption in Down’s syndrome was 4X greater when absorbed as an antacid, and 6X higher in the presence of citrate than that seen in controls. Another study found that adding citrate to aluminum hydroxide antacid increased absorption as much as 11X in normal adults. This may be a good reason to not add lemon juice to your tea, since tea is high in aluminum and lemons are high in citrate.

A monocarboxylic acid transporter controls entry of aluminum into the brain. Pyruvate competes with aluminum citrate for entry, thereby providing a way to inhibit brain accumulation of aluminum. Pyruvate, as well as malate, have also been shown to inhibit glutamate toxicity.

**INFLAMMATION, CYTOKINES AND AUTOIMMUNITY**

For many years scientists suspected that the immune
system played an important role in neurodegeneration. It is known that with aging we begin to develop immune complexes to brain components. Some have proposed that this is the function of immune suppression commonly seen with aging, to reduce the immune attack. Studies of Alzheimer’s patients have shown elevated cytokines IL-1B, IL-2, IL-6, S-100 protein, tumor necrosis factor alpha (TNF-alpha), and significant isolated autoantibodies to GM1. One also sees elevated levels of PGD2 and thromboxane B2, both inflammatory cytokines, in Alzheimer’s disease. Autoantibodies have also been described in amyotrophic lateral sclerosis as well.

This immune attack on neurons produces a state of chronic inflammation that generates a constant high level of free radicals. As the damage continues, the p53 gene is activated, leading to apoptosis. Short of actual destruction of the neurons, a reduction in mitochondrial energy generation leads to increased free radical production. In addition, low energy levels make the neurons infinitely more sensitive to the excitotoxic effects of glutamate and aspartate. In fact, in the face of low energy production, even normal levels of glutamate can kill neurons. Glutamate, in turn, stimulates the microglia, the CNS immune cell, to produce even more cytokines, and to release the two excitotoxins glutamate and quinolinic acid. This vicious cycle leads to eventual neuronal cell death.

As we see, glutamate itself can act as the trigger for microglial activation leading to the release of numerous inflammatory cytokines, or some other event may trigger the process, such as a viral infection, Lyme disease organism invasion, or even heavy metal exposure. One factor that may lead to autoimmunity is the prolonged assault of the brain’s cellular components to oxidative stress. Oxidation of the proteins, which alters their structure, can lead to autoimmunity.

Recent studies have shown that activation of the transcription factor NF kappa B plays a major role in neurodegeneration. This transcription factor stimulates the production of various cytokines including IL-1B, IL-2, IL-6 and TNF-alpha, all of which are increased in neurodegenerative diseases. Oxidative stress is a common trigger for NF kappa B activation.

Beta-amyloid has been shown to activate microglia by way of protein kinase C. Studies indicate that beta-amyloid production is increased in the face of activated microglia and that the presence of beta-amyloid is sufficient to maintain chronic brain inflammation. Microglia themselves contain enough glutamate to elicit excitotoxicity. They can also precipitate excitotoxicity by stimulating the release of arachidonic acid. Microglia also contain considerable amounts of the excitotoxin quinolinic acid, which can be released during activation. Quinolenic acid is a metabolic product of serotonin metabolism.

Animal studies have shown that mice with autoimmune disorders have a more rapid decline in aged-related learning than normal animals.

ADVANCED GLYcation END PRODuctS

One of the consequences of a high dietary intake of glucose, and especially fructose, is the glycation of numerous proteins in the cell. When proteins are glycated, that is, combined with sugar molecules, they become significantly more vulnerable to free radical damage and produce advance glycation end products (AGEs) which can interfere with tyrosine and dopa utilization. Elevated levels of AGEs have been found in Alzheimer’s, Parkinson’s disease, and ALS. This is especially so in Parkinson’s disease because of the early fall in cellular glutathione levels.

The problem of large amounts of AGEs is that they signal glia cells to produce superoxide and nitric oxide, a combination that leads to the production of the powerful peroxynitrite radical. Cytokines are also potent stimulators of inducible nitric oxide, and hence peroxynitrite production.

PERoxYNITRITE

As stated, when nitric oxide combines with superoxide it produces peroxynitrite. This free radical is unusual in that it is resistant to most of the common antioxidants, such as vitamin C, vitamin E and the carotenoids. The most powerful scavenger of peroxynitrite is glutathione. When glutathione levels are low, as is seen in Parkinson’s disease as well as Alzheimer’s dementia and ALS, neurons become significantly more vulnerable. Peroxynitrite tends to concentrate in the mitochondria, where it damages enzymes as well as DNA.

These events dramatically interfere with the cell’s ability to produce energy. Neurons and glia are very energy dependent. Virtually every cellular process requires enormous amounts of energy. The brain consumes 20% of the blood’s oxygen and 25% of its glucose, even though it constitutes only 2% of body weight. Even under deep anesthesia, the brain’s metabolism is reduced only 50%.

Several studies have demonstrated elevated peroxynitrite levels in Alzheimer’s disease, ALS and Parkinson’s disease. Damage by peroxynitrite is indicated by the accumulation of nitrotyrosine.

4-HYDROXYNONENAL (4-HNE)

4-hydroxynonenal (4-HNE) is an aldehydic product of lipid peroxidation. While malondialdehyde (MDA) is the most abundant product of lipid peroxidation, 4-hydroxynonenal is the most reactive with proteins. Interestingly, the distribution of damage by peroxynitrite parallels that of 4-HNE.

There is growing evidence that 4-HNE plays a major role in several neurodegenerative disorders, including...
Alzheimer’s dementia, Parkinson’s disease and ALS. In one study of seven Alzheimer’s disease patients, 4-HNE was found to be associated with all amyloid deposits and most perivascular areas (89%).

Another study found increased 4-HNE in several areas of the brain in Alzheimer’s disease, reaching significant levels in the amygdala, hippocampus and parahippocampus, areas of primary damage in the disorder. Elevations of 4-HNE have also been found in the ventricular fluid of Alzheimer’s patients but not in age-matched controls.

The distribution of 4-HNE appears to be dependent on the presence of the APOE genotype. APOE4-possessing subjects demonstrated primary accumulation of 4-HNE in the cytoplasm of pyramidal neurons, while APOE3 genotypes had both astrocytic and pyramidal cell distribution. APOE4 is strongly associated with Alzheimer’s disease as well as a high risk of dementia pugilistica in boxers. It is also known that individuals with APOE4 genotype have impaired antioxidant enzymes, which may be the basis of their increased incidence of neurodegenerative diseases.

Injecting 4-HNE into the brain of rats causes a widespread loss of neurons in the basal forebrain ipsilateral to the injection and a 60 to 80% reduction in choline acetyltransferase seven days post-injection. When FeCl2 is given, it increases the levels of 4-HNE in the brain.

Similar elevation of 4-HNE has been demonstrated in Parkinson’s disease. A study of seven brains of Parkinson’s disease patients, demonstrated immunostaining for 4-HNE in the striatum, but demonstrated the same findings in only 9% of aged-matched controls.

Direct injection of 4-HNE into the substantia nigra of mice caused a dose-dependent depletion of glutathione in the brainstem. Glutathione levels fall early in Parkinson’s disease. 4-HNE has also been shown to rapidly inactivate glutathione reductase, needed to convert oxidized glutathione to its reduced form.

One of the best correlations with cognitive function in Alzheimer’s disease is the synaptic concentration in the brain. 4-HNE has been shown to conjugate to synaptic proteins and to impair transport of both glucose and glutamate. Both result in a significant decrease in cellular production of ATP.

**HOMOCYSTEINE AND OXIDIZED CHOLESTEROL**

While the cardiovascular system has gotten most of the attention as regards homocysteine and cholesterol, the nervous system is also vulnerable to its effects. Both LDL and HDL exist in the brain, with LDL acting as a transporter of cholesterol and phospholipids in the CNS. Receptors for LDL have been located on microvessels, astrocytes, microglia, and neurons. Like LDL in the plasma, brain LDL and HDL can become oxidized, especially in the presence of increased catalytic iron.

It has been shown that oxidized LDL in the striatum enters the neuron and can induce cell death. The mechanism of neuronal injury is closely connected to excitotoxicity since glutamate-blocking drugs, such as MK-801, protect the neuron from oxidized LDL-mediated cell death. Highly oxidized HDL in the brain has also been shown to increase oxidative stress in astrocytes, microglia, and neurons, causing death in the latter. When oxidized lipoproteins exist in the presence of glutamate and/or amyloid, neuronal killing is enhanced. Oxidized LDL is toxic to motor neuron cells, possibly linking it to amyotrophic lateral sclerosis. Antioxidants, just as in the case of plasma lipoproteins, reduce oxidized LDL and HDL neurotoxicity.

Homocysteine has been strongly associated with cardiovascular disease, even though the mechanism has not been fully elucidated. Less well appreciated is the connection between elevated levels of homocysteine and neurodegeneration. Several recent studies have shown a strong correlation between homocysteine levels and incidence of Alzheimer’s disease. Rarely discussed is the fact that homocysteine is an excitotoxin, as are homocysteic and homocysteine sulphinic acid, two of its metabolic breakdown products.

These excitotoxins act at the N-methyl-D-aspartate (NMDA) receptor, triggering the entry of excessive amounts of calcium into the neuron, leading to numerous destructive reactions including the generation of peroxynitrite, 4-HNE, hydroxyl and peroxyl radicals, and activation of the eicosanoid cascade. Whether lowering homocysteine levels will reduce Alzheimer’s disease is as yet unknown. We do know that folate, pyridoxine, and antioxidant vitamin deficiencies are common in Alzheimer’s disease patients.

Several studies have shown low levels of vitamin B12 as well. It is important to appreciate that the classical hematological signs of B12 deficiency, macrocytosis and hypersegmented neutrophils, are usually absent in these patients. While homocysteine levels were found to be consistently elevated in Alzheimer’s patients, nutritional deficiency was not confirmed using retinol binding protein (RBP). This indicates an impaired cobalamin delivery to the tissues, which explains the observed discrepancy between normal serum levels of cobalamin and folate and low tissue metabolic products found frequently in the elderly.

One recent study throws the homocysteine theory into question. Centenarians living in two northern Italian provinces were examined for blood levels of homocysteine, folate and B12. They examined centenarians who were cognitively normal, cognitively impaired, and those with a diagnosis of Alzheimer’s disease. Elevated homocysteine levels were found in 77% of normal, 100% of cognitively
impairment, and 82% of Alzheimer’s patients. Demented centenarians had the lowest folate levels. Low B12 and B6 levels were found in 33% and 66% respectively of all centenarians regardless of cognitive status.

There are several explanations for these negative findings. First, the study was based on blood levels of the vitamins, and as we have seen, there is little correlation between blood levels and tissue levels of these three vitamins. As for the lower level of homocysteine seen in the centenarian Alzheimer patients, perhaps it was secondary to metabolic burnout, something we see in the case of glutamate as well.

Homocysteine is known to elicit apoptosis quite rapidly when hippocampal neurons are exposed to this amino acid. The mechanism includes DNA strand breaks with activation of poly-ADP-ribose polymerase (PARP) which depletes nicotinamide adenine dinucleotide (NAD). This in turn leads to mitochondrial dysfunction, oxidative stress and caspase activation. In essence it markedly enhances the vulnerability of hippocampal neurons to excitotoxic and oxidative injury.

ENERGY PRODUCTION, EXCITOTOXICITY, AND FREE RADICALS

There is an intimate connection between energy production, excitotoxicity, and free radicals. It has been known for some time that impaired mitochondrial energy production can lead to dramatic increases in free radical production, and that reduced neuronal energy production significantly increases the neuron’s sensitivity to excitotoxicity. In fact, under such conditions even normal concentrations of extracellular glutamate can trigger excitotoxic reactions. Further, an increase in free radical production, either as reactive oxygen or reactive nitrogen species, increases the release of glutamate from the astrocyte. Glutamate in turn increases free radical production, which further reduces energy production. This vicious cycle continues until the p53 gene is activated and apoptosis ensues. The neurons are destroyed by necrosis as well. When elevated iron levels are present, as it does in all neurodegenerative disorders, free radical generation reactions are accelerated.

Another result of increased free radical generation is its effect on the blood-brain barrier system. This gatekeeper normally prevents or slows the passage of harmful molecules into the brain’s environment. Unfortunately, the system is not perfect. Recent studies have shown that free radicals can open the barrier, allowing harmful compounds inside, including the excitotoxins glutamate and aspartate. Evidence also indicates that the barrier contains glutamate receptors and that glutamate itself can open the barrier. This means that elevated levels of blood glutamate can open the barrier, further elevating the level of this powerful excitotoxin in the extracellular space.

Related to energy deficits in neurodegeneration is the finding that the glucose transporter is impaired in Alzheimer’s disease secondary to alteration in the brain’s vasculature. In addition, glutamate itself can impair glucose entry into the brain.

So we see that there is an intimate connection between glutamate, free radicals, energy production, and the widespread destruction of neurons. Because neurons differ in the types of neurotransmitter receptors present on their membranes, some will be more sensitive than others. This accounts for the difference in pathological presentation.

Reduced energy production has been demonstrated in all four major neurodegenerative disorders: Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and amyotrophic lateral sclerosis. In each instance, specific enzyme deficiencies are present. In Parkinson’s disease, complex I is deficient. In Alzheimer’s disease complex I, IV and pyruvate dehydrogenase complex are all deficient.

MAGNESIUM

Magnesium plays a special role in excitotoxicity and free radical generation. The magnesium receptor is located near the calcium channel on neurons possessing the NMDA receptor, which regulates calcium entry into the neuron. As we have seen, excessive intracellular calcium can trigger destructive reactions involving nitric oxide synthase induction, arachidonic acid release from the membrane with eicosanoid activation, and changes in mitochondrial function. Magnesium can block calcium entry as long as the neuron is not firing. It has been demonstrated that low magnesium levels greatly enhance the neuron’s sensitivity to glutamate, again, where even normal levels can be excitotoxic.

Other studies indicate that magnesium plays a major role in preventing free radical accumulation. Cells isolated in a low magnesium environment not only generate more free radicals but are twice as sensitive to free-radical-induced cell death as cells with normal magnesium levels. In addition, hypomagnesemia lowers the cell’s glutathione level and increases its cytokine level, which, as we have seen, can inhibit glutamate uptake, thereby increasing free radical generation that leads to excitotoxic neuron death.

Both experimental and clinical studies have demonstrated significant neuroprotective effects of elevated magnesium levels. An additional benefit is that magnesium also inhibits the entry of oxidized lipids into the endothelial cells of blood vessels.

INHIBITORS OF GLUTAMATE UPTAKE: THE GLUTAMATE TRANSPORT PROTEINS

Because even small concentrations of extracellular glutamate can trigger neuron destruction, it is carefully reg-
ulated by a special re-uptake system. This system consist of approximately five different transport proteins, EAAT 1-5, whose job it is to bind to glutamate, transport it to the astrocyte and transfer it into the intercellular compartment. Once this is done, the glutamate is converted into glutamine and stored until needed. The distribution of the different types of transport proteins is brain specific.113 EAAT 1-5 are found in the retina.

There is growing evidence that abnormalities in these transport proteins play a major role in many neurodegenerative diseases. This is best demonstrated in amyotrophic lateral sclerosis (Lou Gehrig disease).114 There is some evidence that a similar process operates in Alzheimer’s dementia and Parkinson’s disease.115,116

We now know that several events and compounds (such as viral infections,117 hereditary SOD1 mutants in ALS,118 oxidative stress119 and exposure to mercury) can trigger excitotoxicity by inhibiting the glutamate transporters.

Mercury is a very potent inhibitor of glutamate transporters. In one study a mercury dose as low as 10(-5)M was found to be inhibitory.120 Mercury is unique, since no other metal tested, including aluminum, lead, cobalt, strontium, cadmium and zinc, had any effect on glutamate transport.121

What makes this important is the ubiquitous nature of mercury exposure. The number one source for humans is dental amalgam.122 Other sources include industrial fumes, coal burning, contaminated fish and contaminated lakes and streams. Dental amalgam is composed of 50% mercury, which has been shown to vaporize in the mouth, especially in the presence of heat. Drinking hot liquids and chewing has been shown to increase the release of mercury vapor from amalgams at levels 3X higher than normal for 90 minutes.123,124 Over 80% to 90% of this vapor is absorbed into the circulation. Because of its fat solubility, mercury accumulates in high levels in the nervous system and is very difficult to remove.125 Mercury is also a potent stimulus for the production of free radicals and it inhibits numerous enzymes, including the antioxidant enzymes.126

As I demonstrated earlier, a key player in excitotoxicity and neurodegenerative diseases is 4-hydroxynonenal (4-HNE), a product of lipid peroxidation, and a potent inhibitor of glutamate transport proteins.127 This once again forges a strong connection between excitotoxicity and oxidative stress. Inflammatory cytokines, such as IL-1β, IL-2, IL-6 and TNF-alpha, can also inhibit the glutamate transporters, likely via increased lipid peroxidation and production of excessive amounts of 4-hydroxynonenal.

So we see that anything that increases oxidative stress in the nervous system over a long period of time will increase the risk of neurodegeneration. This explains why so many seemingly unrelated factors – such as viral infections, trauma, pesticide exposure, hereditary enzyme defects, and exposure to mercury, aluminum, fluoride and iron can play a role in producing the neurodegenerative diseases. They all increase oxidative stress, inhibit glutamate transport, activate microglia, and trigger excitotoxicity.

THE USE OF NUTRACEUTICALS AND PLANT EXTRACTS IN PREVENTING AND TREATING NEURODEGENERATION

A principal cause of neurodegeneration, oxidative stress, can be substantially reduced by the consumption of various nutraceuticals.128 Flavonoids are powerful, versatile antioxidants whose potency is enhanced when combined with vitamins and minerals.

ANTIOXIDANT EFFECTS

Most are familiar with the antioxidant effects of vitamins such as the tocopherols and tocotrienols, ascorbate, vitamins D and K, and the minerals zinc, magnesium and selenium. As efficient as these antioxidants are, especially in combination, they are ineffective against some of the major free radicals and reactive nitrogen species. A study of Alzheimer’s and multi-infarct dementia patients found that both were more often deficient in vitamin E and beta carotene than matched controls.129 Only the Alzheimer’s patients were deficient in vitamin A.

A number of antioxidants have been shown to inhibit glutamate-induced cytotoxicity (excitotoxicity) including vitamin E, Ginkgo biloba extract, pycnogenol, N-acetyl L-cysteine, alpha lipoic acid, DHLA, and individual flavonoids.130 Of even greater interest is the finding that not only can flavonoids protect DNA from oxidative injury, they initiate fast chemical repair of DNA as well.131

As we have seen, peroxynitrite plays a central role in neurodegeneration because of its toxic effects on mitochondrial enzymes and mitochondrial DNA. While the antioxidant vitamins are generally ineffective in inhibiting these radicals, flavonoids are quite efficient. In fact, a study of the scavenging capacity of flavonoids as compared to a standard peroxynitrite scavenger, ebselen, found that the flavonoids were 10X more potent.132

Researchers recently found that teas, both black and green, have peroxynitrite scavenging ability equal to that of red wine polyphenols.133 In their study, lipopolysaccharide-induced nitric oxide synthase (iNOS) activity was dramatically reduced, most likely by epigallocatechin gallate, but the mixed theoflavins from black tea were also potent inhibitors. Peroxynitrite is formed when nitric oxide is produced in excess. The black tea component, theaflavin digallate, was also found to decrease superoxide production in macrophages and to chelate iron to a significant degree, moreso than green tea components.134

Curcumin, from the spice turmeric, is also a potent inhibitor of peroxynitrite and lipid peroxidation.135 By
enhancing the production of glutathione, curcumin further protects neurons from peroxynitrite, as well as numerous other free radical oxygen and nitrogen species. Alpha lipoic acid and its reduced form, dehydroalphaic acid (DHLA) also enhances cellular glutathione production. Studies have shown alpha lipoic acid to increase glutathione levels from 30 to 70% higher than normal. Hydroxytyrosol, found in extra virgin olive oil, is highly protective against the peroxynitrite radical as well.

Ubiquinone (coenzyme Q10) may act as a significant antioxidant in biological systems. It may do this by regenerating vitamin E. In turn, alpha-lipoic acid can increase the level of ubiquenol in the face of oxidative stress. So we see a complex interplay of the antioxidants that allows them to be regenerated for further use.

The reduced form of alpha-lipoic acid, DHLA, has the greatest versatility in neutralizing free radicals. DHLA can neutralize the hydroxyl radical, singlet oxygen, hypochlorite, NO radicals, superoxide, peroxyl radicals and $H_2O_2$, whereas alpha-lipoic acid cannot neutralize superoxide or peroxyl. In mammalian cells alpha-lipoic acid is rapidly converted to DHLA. Both alpha-lipoic acid and DHLA have been shown to be protective against NMDA and malonic acid-induced striatal lesions in the brain, reducing the size of the lesion by 50%. This would be important in preventing the oxidative stress lesion responsible for Parkinson’s disease.

Melatonin is also gaining interest as a powerful neuroprotectant. It has been shown to react with the hydroxyl radical, hydrogen peroxide, singlet oxygen, peroxynitrite, nitric oxide, and hypochlorous acid. In addition, it stimulates the production of the antioxidant enzymes: superoxide dismutase, glutathione peroxidase and glutathione reductase. In a test using dopamine, neuronal cell cultures grown in vitro in a medium without supporting growth factors, all of the cells were dying within a short period of time. When melatonin was added to the suspension, nearly all of the dying cells were rescued, including tyrosine hydroxylase positive DA neurons.

PREVENTION OF LDL OXIDATION

As we have seen, LDL and HDL exist in the brain and when oxidized can induce neuron cytotoxicity. It is interesting to note that oxidized LDL cytotoxicity acts through the NMDA receptor by way of the excitotoxic mechanism. One major system preventing lipoprotein oxidation is the arrangement of tocopherol molecules within the LDL and HDL units. The LDL structure contains six tocopherol molecules. As with all tocopherols, those in lipoproteins can become oxidized when exposed to excessive oxidative stress. Regeneration of embedded tocopherol depends on other antioxidants, such as the carotenoids, ascorbate, alpha-lipoic acid, DHLA, coenzyme Q10, and the flavonoids.

Dietary supplementation with alpha-tocopherol has been shown to reduce the oxidative modification of LDL, a reduction even greater in diabetics. Ascorbate has also been shown to be an effective inhibitor of LDL oxidation, and combined with alpha-tocopherol, has reduced the susceptibility of LDL to oxidation at all concentrations of copper tested.

Coenzyme Q10 has been shown to significantly reduce the oxidizability of LDL in the face of aqueous free radical generation at a dose of 300 mg a day in humans. Numerous flavonoids have been shown to reduce LDL-oxidizability including red wine polyphenols (catechins), myricetin, quercetin, epigallocatechin gallate, epicatechin and rutin.

I would caution that drinking red wine for health benefits may be more hazardous because of the high concentration of fluoride in California wines and the use of sulfites in most wines. The sulfite connection is especially strong because of the observed enhancement of neuronal toxicity when sulfite exists in the presence of peroxynitrite, especially when combined with glutathione depletion, as is seen in Parkinson’s disease. Finally, the alcohol itself is particularly toxic to neurons. A recent study found a graded deleterious effect of alcohol on antioxidant levels within synaptosomes and neuronal mitochondria. There was also a dose-dependent increase in lipid peroxidation.

A recent study found that the most effective protection against oxidized LDL-induced cytotoxicity was from cyanidin, epicatechin and kaempferol, with 80% protection. One of the most effective flavonoids, epicatechin, was 10X more efficient in protecting neurons under these conditions than ascorbate. Pretreatment with taxifolin, apigenin and naringenin enhanced the toxic effect of oxidized LDH in vitro, even though they were not neurotoxic alone. This study demonstrates the usefulness of selected flavonoids as powerful neuroprotectants under conditions of oxidative stress.

The double advantage to lowering LDL and HDL oxidation is a reduction in both direct neurotoxicity of oxidized LDL and HDL, and the prevention of atherosclerotic cerebrovascular disease.

INFLAMMATION, CYTOKINES AND NUTRACEUTICALS

All of the major neurodegenerative disorders are associated with microglial activation and excessive production of cytokines IL-1beta, IL-6, and TNF-alpha. This inflammatory process involves overactivation of the eicosanoid system through activation of phospholipase A2 and the release of arachidonic acid from the membrane. This in turn is acted on by lipoxigenase and cyclooxygenase with the production of numerous pro-inflammatory leukotrienes and prostaglandins. Excitotoxins also induce interleukin-1beta both in microglia and astrocytes.
Attempts to reduce neurodegeneration have recently focused on ways to inhibit this series of pro-inflammatory reactions. In one transgenic Alzheimer’s mouse model study, it was found that ibuprofen significantly reduced IL-1β and glial fibrillary protein levels and reduced the total number of amyloid deposits.155

Another cytokine of importance in neurodegeneration is tumor necrosis factor-alpha (TNF-alpha), which is elevated in Alzheimer’s disease, Parkinson’s disease, and ALS. It is known that (-) epigallocatechin gallate inhibits the production of TNF-alpha by modulating the pro-inflammatory transcription factor NF kappa B. Other flavonoids, such as curcumin and quercetin,156,157 can also modulate NF kappa B. In one study, all flavonones tested protected cells against TNF cytotoxicity, with eriodictyol being most potent.158 Apigenin markedly enhanced the cytotoxicity of TNF. Zinc has been shown to markedly inhibit apoptosis induced by TNF.159

Critical to the neurodegenerative process is the inflammatory cascade, which involves numerous cytokines, eicosanoids, and other immune factors. We know there is an intimate connection between excitotoxicity and the inflammatory cascade in cells. The inflammatory cascade can be blocked or reduced at any one of these levels.

Since NF-kappa B transcription factor plays a major role in CNS inflammation, blocking its activity can reduce inflammation, making quercetin, apigenin, and curcumin especially useful in this regard.160 Some flavonoids inhibit the release of arachidonic acid from the membrane.161 These include amentoflavone (Ginkgo leaf), quercetagetin-7-O-glucoside, apigenin, fisetin, kaempferol, luteolin, and quercetin.

Apigenin, genistein, and kaempferol have been found to be potent inhibitors of the COX-II enzyme, which is responsible for inflammatory reactions.162 Curcumin is also a potent inhibitor of the COX enzymes and is equal in potency to NSAIDs.163 They also inhibit inducible nitric oxide synthase (iNOS), which triggers the production of the powerful and destructive peroxynitrite radical. Quercetin, which significantly inhibits COX enzymes, is a more potent inhibitor of lipoxygenase (LOX).164 Interleukin-12 also plays a vital role in inflammation; it is potently inhibited by the flavonoid curcumin. In addition, curcumin has been shown to potently inhibit prostaglandin activation in cases of toxic damage to the brain by alcohol.165

So we see that flavonoids act at multiple sites to inhibit the destructive reactions precipitated during neurodegeneration, including excitotoxicity, microglia activation, glutamate transporter inhibition, transitional metal activation of free radicals, and direct inhibition of the inflammatory processes. Finally, there is some evidence that one herb, Ashwagandha, can act as an immune modulator.166 That is, it can suppress an overactive immune response.

IMPROVING ENERGY PRODUCTION

As we have seen, cellular energy production plays a pivotal role in protection against excitotoxicity. At present, there are numerous ways to improve mitochondrial energy generation. Of great importance in neurodegenerative disorders is the ability to bypass blocks in the electron transport chain, as is seen in both Parkinson’s and Alzheimer’s disease. For example, coenzyme Q10, succinate, and β-hydroxybutyrate have all been shown to bypass complex I defects.167 Pyruvate and malaate have been shown to protect cortical neuron cultures from excitotoxic cell death following exposure to glutamate, mostly by increasing cell energy generation.168 Supplementation with creatine also protects against excitotoxic injury.169

Many other vitamins and minerals play a role in cellular energy production, including magnesium, thiamine, riboflavin, niacinamide, menadione, tocophersols, folate, ascorbic acid, succinate, acetyl-L-carnitine, and alphalipoic acid.170 Acetyl-L-carnitine has been shown to partially restore mitochondrial function in elderly rats,171 while treatment of old rats with alpha-lipoic acid has been shown to improve mitochondrial energy production and increase their metabolic rate.172

Protecting the cell, and its mitochondria, from the effects of free radicals plays a key part in preserving cellular energy production. Remember, mitochondrial DNA is 10X more sensitive to free radical injury than is nuclear DNA.

DIRECT BLOCKING OF EXCITOTOXICITY

Several nutrients can directly block the excitotoxic process itself. For example, methylcobalamin has been shown to block the NMDA glutamate receptor on the neuron.173 Pycnogenol has been shown to inhibit the cytotoxic effects of amyloid β-protein and to protect hippocampal neurons from high concentrations of glutamate.174

The natural product vinpocetine not only increases cerebral blood flow but also inhibits glutamate receptors and regulates Na+-channels, offering potential benefits against neurodegenerative disorders.175 Low doses of vitamin D have been shown to protect neurons by down-regulating L-type voltage-sensitive calcium channels, thereby protecting hippocampal neurons in culture from excitotoxicity.176

Another way flavonoids may help prevent excitotoxic lesions in the nervous system is by reducing histamine release and activity. A recent study found that activated mast cells in the CNS increased excitotoxic injury 60% by potentiating receptor-mediated events at the NMDA receptor.177 Vitamin C inhibits the release of histamine from mast cells, and quercetin blocks the histamine receptor. While neuroprotection by quercetin’s action on brain histamine has not been demonstrated, it deserves a closer look.

Combining nutrients appears to offer more neuropro-
tective than using single agents. In one study, combining coenzyme Q10 and nicotinamide significantly protected striatal neurons in vivo, against excitotoxic destruction, while CoQ10 alone was not protective. Likewise, vitamin C and alpha-tocopherol used in combination inhibited lipid peroxidation in mice brains significantly better than either agent used alone.

OTHER NEUROPROTECTIVE NUTRACEUTICALS

Docosahexaenoic acid (DHA) is essential for the normal development and function of the infant brain and for the maintenance of the adult brain. It should be remembered that the adult brain is constantly remolding itself, a process called plasticity. This remodeling process primarily involves synaptic reorganization. Considerable scientific literature confirms the importance of docosahexaenoic acid (DHA) in this process. Because of the widespread consumption of processed foods, deficiencies of DHA are common. The ratio of N-6 fats to N-3 fats is now 25:1 when it should be 5:1. DHA reduces the risk of neurodegeneration not only by improving cerebral plasticity, but also by reducing inflammation. Without antioxidant supplementation, DHA alone can increase free radical production and lipid peroxidation.

A fairly recent study found significant abnormalities in amino acid metabolism in Alzheimer’s disease patients. Significantly reduced plasma amino acids included tryptophan and methionine. Excessive supplementation with serotonin precursors could potentially lead to increased excitotoxic injury due to a buildup of the serotonin metabolic product quinolinic acid, a known excitotoxin. Taurine has been shown to have neuromodulatory effects in the CNS and to regulate cell volume.

Another study found low levels of s-adenosylmethionine in Alzheimer’s patients. A postmortem study of 11 patients with Alzheimer’s disease found low levels of s-adenosylmethionine in all areas of the brain as compared with matched controls. In this same study, normal levels were found in cases of Parkinson’s disease, demonstrating that a low level of s-adenosylmethionine was not merely an epiphenomenon of neurodegeneration. No studies have been done to supplement Alzheimer’s patients with s-adenosylmethionine. It may be that these low levels merely reflect a deficiency in folate, pyridoxine, and cobalamin, which is known to occur in Alzheimer’s disease. Chronic folate deficiency has been associated with cancer and may also have a significantly deleterious effect on brain function as well, especially when combined with prolonged injury by reactive oxygen and nitrogen species.

Growing evidence indicates that several hormones can protect neurons from numerous types of injury including neurodegeneration. The earliest attention was given to estrogen hormones and their ability to attenuate the symp-
ascorbate. In this study, plasma vitamin C levels fell in proportion to the severity of the disease.

Two herbs, Ginkgo biloba and Panax ginseng, have shown both clinical and experimental promise in preventing and treating neurodegenerative disorders. In a double-blind, randomized, placebo-controlled clinical study involving 309 patients with mild to moderate Alzheimer’s dementia, researchers found that moderately low doses of Ginkgo extract (EGb 761) could slow the course of the disease and improve mental functioning in a substantial number of patients. Another study using 240 mg of Ginkgo biloba extract also found substantial benefit in Alzheimer’s patients with a wide margin of safety.

In another trial, 256 healthy, middle-aged volunteers were given either 160 mg or 320 mg of a mixture of standardized Ginkgo biloba and Panax ginseng for 14 weeks. At the end of the trial, substantial improvements in both working and long-term memory were seen, an effect that lasted beyond the two-week washout at the end of the trial.

Studies have shown that Ginkgo biloba extract can protect neuron membranes against hypoxia-related breakdown, something that probably plays a vital role in Alzheimer’s disease. By its powerful antioxidant effects, Ginkgo biloba demonstrates an ability to preserve mitochondrial function in aged animals, which, as we have seen, is vital to preventing accumulative excitotoxic-free radical injury.

Ginsenosides Rb1 and Rg3 have been found to significantly protect cultured rat cortical neurons from neurodegeneration precipitated by excess glutamate. Important in preventing Alzheimer’s-type neurodegeneration, Rb1 increases choline acetyltransferase in the basal forebrain and nerve growth factor in the hippocampus. This also explains the finding of improved memory function in scopolamine-treated young and old rats treated with ginsenosides Rb1 and Re.

Finally, one naturally found substance with much promise is GM-1 ganglioside. In experiments using monkeys treated with 1-methyl-1,4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce Parkinson’s disease, GM-1 ganglioside was found to exert a neurotrophic effect on the surviving neurons in the substantia nigra. In another study, GM-1 ganglioside was found to protect against motor neuron death in rats, and to reduce by half the number of degenerating fibers in their spinal cords following injury.

**REDUCING MERCURY TOXICITY**

Mercury is one of the most neurotoxic elements found in nature. Unfortunately, millions of people are being put at unnecessary risk by having dental amalgam placed in their teeth as a restorative. Amalgam contains approximately 50% mercury. Another common source is thimerosal, a preservative in some vaccines. With tens of millions of babies and children being vaccinated each year with up to 33 vaccinations before age two, a frightening health disaster is in the making. There is no known safe level of mercury.

The ability of our cells to resist toxins depends on their overall health and especially their antioxidant capacity. It has been demonstrated that one’s sensitivity to mercury is directly related to tissue levels of alpha-tocopherol and selenium, especially in the nervous system. Zinc may protect the nervous system from mercury toxicity via its role in the production of metallothionein.

While no one has tested the ability of plant flavonoids to chelate mercury, several, including curcumin, hesperidin, quercetin, tea catechins, and rutin, have been shown to have powerful chelating ability for iron and copper.

Mercury has been shown to powerfully bind with citrate and malate to form a harmless compound. In addition, both easily penetrate the blood-brain barrier. Removal of mercury from the brain is a difficult and slow process, but by utilizing these organic compounds one can significantly reduce its toxicity. Combining magnesium to malate and citrate would further reduce mercury toxicity by their combined ability to reduce NMDA activity, increase cellular glutathione levels and reduce free radical injury.

Garlic extract has also been shown to efficiently remove mercury from the brain. In fact, it is almost as efficient as 2,3-dimercaptoposuccinic acid (DMSA). In addition, garlic binds and removes mercury within the GI tract, the major reservoir for mercury. The active principle may be selenium.

High doses of alpha-lipoic acid, a powerful and versatile antioxidant, are also an efficient chelator of mercury. It easily penetrates the blood-brain barrier and has been shown to reverse the age-related changes in long-term potentiation (LTP) responsible for laying down memory.

Since mercury in brain tissue is associated with dramatic increases in free radical formation, all preceding comments concerning antioxidant supplements and flavonoids apply. In addition, recent studies have directly linked neuron exposure to mercury with the formation of $\beta$-amyloid as well as hyperphosphorylation of the tau protein. This is most likely related to both free radical generation and direct effects of mercury on amyloid $\beta$ protein and tau phosphorylation.

Finally, exposure to mercury induces autoantibodies to neurotypic and gliotypic proteins, common to all three of the major neurodegenerative diseases. Mercury exposure has been shown to increase the number of microglia cells in the brain, wherein the mercury accumulates. Again, all nutritional factors affecting the immune response would apply here.
SUMMARY

We have seen that neurodegeneration is a complex process involving several cellular systems including free radical generation, the antioxidant network, eicosanoid activation, lipid peroxidation products that inhibit glutamate re-uptake, a loss of cellular energy production, and the buildup of advanced glycation end products. All of these processes are connected to the excitotoxic reaction.

That chronic inflammation may be the central cause of neurodegenerative diseases is only one part of the puzzle. Overactivation of the glutamate receptors will trigger activation of the microglia, leading to the immune–cytokine activation process, and will trigger a tremendous generation of reactive oxygen and nitrogen species. This in turn leads to impairment of the energy-generating system, primarily by the action of free radicals (peroxynitrite and hydroxyl ions) on the mitochondrial DNA and electron transport system enzymes.

While different triggering events can initiate these destructive processes at any level, all result in glutamate receptor overactivity and initiation of the excitotoxic process. We have seen that several nutraceuticals can act at one of multiple levels in this destructive process. The flavonoids, in fact, operate simultaneously at many arms of the excitotoxic reaction.

One key to preventing neurodegeneration is to maintain the cell’s energy supply. Events that interfere with cellular energy production, no matter the cause, will result in neurodegeneration. Nutraceutical research remains an area of much promise in conquering this dreaded process.

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Breast Cancer, Hormone Replacement Therapy (HRT), and Diet

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Breast cancer is second only to lung cancer as the most common cause of cancer mortality in women in the United States. Further, in 2000 alone, there were a predicted 182,000 new cases of breast cancer diagnosed and 40,800 associated female deaths in the U.S. as a result of this disease. A woman's chance of developing breast cancer significantly increases with age (Table 1). As a woman ages, she will naturally approach menopause and the cessation of ovarian function, increasing her chances of taking hormone replacement therapy (HRT) to reduce symptoms commonly associated with menopause and to prevent the onset of heart disease. Disturbingly, HRT also increases a woman's risk of breast cancer, sometimes by more than 50%.2-6

Two small studies, not adequately controlled for confounding variables, showed some promise that combined HRT was protective against breast cancer risk.8,9 The much larger Nurses' Health Study10 was one of the first studies to provide reliable data that showed that combined therapy actually increases a woman's risk of breast cancer! Persson et al. studied the risk of breast and endometrial cancer in a cohort of 11,231 Swedish women prescribed different replacement hormone regimens and concluded that long-term recent use of estrogen-progestin combined replacement therapy may increase the risk of breast cancer.11 Further, they concluded that exposure to estrogen alone substantially elevates the risk of endometrial cancer, an increase that can be reduced or perhaps avoided by adding progestins. Remarkably, this study found that for 6 or more years of current or recent use of combined HRT, a woman's risk of breast cancer is increased by 70% as compared to the risk associated with estrogen alone, RR = 4.2 (95% CI 2.5-8.4). Considering the fact that in December of 2001 a study of more than 200,000 women was published in the Journal of the American Medical Association12 that found those women who used estrogen replacement therapy (ERT) for a decade or longer also had at least a 51 percent greater chance of dying from ovarian cancer, and that the risk persisted for 29 years after cessation of use, these findings may leave a woman concerned about the impact of HRT on long-term health. While ERT is a regimen that was for the most part discontinued in the late 1970s, one wonders if, 30 years from now, other risks associated with HRT may surface from the research. The results of an HRT and breast cancer study published in the Journal of the American Medical Association13 in 2000 provide further confirmation of the breast cancer risk associated with menopausal estrogen and estrogen-progestin replacement therapy. While HRT appears to increase breast cancer risk, there is evidence suggesting that diet, particularly the use of unprocessed whole foods and herbs, meaning those natural raw materials that have not been fractionated to isolate and/or potentate one or several compounds, and the use of

<table>
<thead>
<tr>
<th>Age</th>
<th>Chance of developing breast cancer</th>
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<tbody>
<tr>
<td>By 30</td>
<td>1 out of 2,212</td>
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<tr>
<td>By 40</td>
<td>1 out of 235</td>
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<tr>
<td>By 50</td>
<td>1 out of 54</td>
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a calorie restricted diet, may play a role in diminishing breast cancer risk. This paper represents a review of evidence supporting this notion.

**DIET AND BREAST CANCER**

Migration studies have shown that people who move from geographical areas with low breast cancer risk to areas with high risk acquire the breast cancer rate of the host country within two generations, and the breast cancer rates are steadily rising in countries with previously low rates. These results suggest that environmental factors, most likely dietary patterns, play a major role in breast cancer etiology.

Analysis of an Italian case-control study conducted from 1991-1994 by Favero et al. on a large sample of women (2,569 women with breast cancer and 2,588 controls) on dietary factors affecting breast cancer risk revealed direct associations between risk and consumption of such foods as bread and cereals, sugar and candy, and pork and processed meats, but inverse associations with the intake of raw vegetables and fish (Table 2). However, cooked vegetables, fruit, and red meat were among the foods that did not seem to have an effect on risk.

Starch, saturated fat, and alcohol were significantly and positively associated with cancer risk while unsaturated fat was inversely associated. Interestingly, olive oil...

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Table 2. Odds ratios (OR) of breast cancer among 2,569 cases and 2,588 controls by intake quintile of selected food groups. Italian (Favero) study, 1991-1994.

<table>
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<tr>
<td></td>
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<td>2</td>
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<tr>
<td>Bread and cereal dishes</td>
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<td>1.11</td>
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<td>OR (95% CI)</td>
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<td>(1.0-1.5)</td>
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<td>Poultry</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Upper limit (servings/week)</td>
<td>1</td>
<td>0.86</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.7-1.1)</td>
<td>(0.7-1.0)</td>
</tr>
<tr>
<td>Pork and processed meats</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Upper limit (servings/week)</td>
<td>1</td>
<td>0.92</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.8-1.1)</td>
<td>(0.8-1.2)</td>
</tr>
<tr>
<td>Fish</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Upper limit (servings/week)</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.9-1.3)</td>
<td>(0.8-1.2)</td>
</tr>
<tr>
<td>Raw vegetables</td>
<td>4.9</td>
<td>7.4</td>
</tr>
<tr>
<td>Upper limit (servings/week)</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.8-1.1)</td>
<td>(0.7-1.0)</td>
</tr>
<tr>
<td>Sugar and candies</td>
<td>7.0</td>
<td>18.5</td>
</tr>
<tr>
<td>Upper limit (servings/week)</td>
<td>1</td>
<td>1.31</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(1.1-1.6)</td>
<td>(1.1-1.6)</td>
</tr>
<tr>
<td>Specific seed oils</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>Upper limit (g/day)</td>
<td>1</td>
<td>1.01</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.8-1.2)</td>
<td>(0.8-1.1)</td>
</tr>
<tr>
<td>Mixed seed oils</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Upper limit (g/day)</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>(0.8-1.1)</td>
<td>(0.8-1.1)</td>
</tr>
</tbody>
</table>

1 Reference category  2 Chi-square test  3 Safflower, maize, peanut, or soya.
showed a protective effect but did not show statistical significance. Beta-carotene, vitamin E, and calcium-rich foods are inversely correlated with risk of breast cancer when controlled for overall energy intake and confounding intakes of other micronutrients (Table 3).

In Italy, pasta and bread comprise a large percentage of the dietary starch and energy, and it is often in the form of very refined grain products. Processed meats are quite common in the Italian diet as prosciutto, salami, and sausage, and these were found to be particularly detrimental with regard to breast cancer risk.

Seed oils and fish in the diet correspond to significantly decreased cancer risk. These foods are especially high in omega-3 fatty acids, supporting growing evidence that this type of fat bestows protection from breast cancer.

Raw vegetables are an important source of dietary fiber and may decrease cancer risk by helping to curb caloric (energy) intake.

The Favero study took into account strong intercorrelations between various macronutrients in the diet, sometimes neglected in other studies. It emphasized a whole food approach by examining not just isolated macronutrients, but macronutrients within a dietary framework. For example, they found in their sample that starch intake correlated negatively with saturated fat intake. This is an interaction that may influence risk. Polysaturated fat intake was strongly positively correlated with raw vegetable intake and both of these factors together lower breast cancer risk. Interestingly, the inverse relationship of breast cancer risk to calcium intake was very strong and is supported by several case control studies. There was also a high correlation between starch and energy intake (Pearson r=0.80). In general, the researchers concluded that energy intake may be related to cancer risk by increasing oxidative metabolism and free radical generation in the body. In keeping in line with a free radical metabolic hypothesis of cancer initiation, dietary restriction may offer a means of reducing cancer risk.

**DIETARY RESTRICTION AND CANCER**

Caloric restriction without malnutrition (cutting daily caloric intake by 30-50% while maintaining a nutritionally balanced diet) is the only proven method to date that slows the human aging process and extends lifespan, while improving health and well-being. Researchers have been stating this once theoretical notion as fact for well over 60 years. While human trials are lacking to support the connection between breast cancer risk and the use of a caloric restricted diet, particularly those that address confounding variables such as ethnicity, BMI, exercise habits, and specific BRCA mutations, the available relevant data does offer some groundwork upon which future researchers may build a greater understanding of this method.

**POSSIBLE MECHANISMS OF ACTION**

Dietary restriction reduces the number of reactive oxidant molecules in the body. While numerous mechanisms have been proposed for the observed long-term health benefits of caloric restriction, the free radical theory is the most widely accepted. Dietary restriction modulates many key aspects of free radical metabolism, including free radical generation, lipid peroxidation, DNA damage, and cytosolic cellular defense systems. This theory offers a solid explanation for the following biochemical and physiologic phenomena:

---

**Table 3.** Odds ratios (OR) of breast cancer among 2,569 cases and 2,588 controls by intake quintile of selected micronutrients. Italian (Favero) study, 1991-1994.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Quintile</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-carotene</td>
<td>1</td>
<td>2778</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3666</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4566</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5852</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1</td>
<td>7.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.82</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13.43</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Calcium</td>
<td>1</td>
<td>671</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>871</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1050</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1297</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Reference category  
2 Chi-square test
• The inverse relationship between basal metabolic rate and average lifespan in mammals
• The connection between increased incidences of degenerative diseases in the latter part of life, and the cumulative effects of oxidative damage over time
• The demonstrated health benefits of dietary restriction
• Greater longevity of females
• The increase in autoimmune dysfunctions with age

DIETARY RESTRICTION AND FREE RADICALS

In diet restricted rats, free radical damage as measured by thiobarbituric acid-reactive material and lipofuscin accumulation in the liver was much lower than in rats fed ad libitum. In liver tissue, the expression of superoxide dismutase and catalase typically decrease with age in ad libitum fed rats. However, in diet restricted rats these antioxidant enzymes were significantly elevated even at 21 and 28 months of age. Glutathione peroxidase was also elevated at 28 months of age. This result has been supported by additional research in which glutathione reductase, glutathione S-transferase, and catalase enzyme activities were essentially unchanged in diet restricted rats at 24 months, while the levels in ad libitum fed rats steadily declined from 12 to 24 months of age. These studies suggest that the beneficial effects of dietary restriction result from the preservation of antioxidant enzyme activity in animals.

Some researchers argue that dietary restriction inhibits the generation of oxidative molecules and does not directly affect antioxidant enzyme levels. Gong et al. reported an apparent reduction in antioxidant enzyme activity in rat lens and kidney in response to dietary restriction, presumably due to a decrease in substrate molecules. Dietary restriction at various ages in the rat decreases the formation of superoxide and hydroxyl radicals, and hydrogen peroxide in liver microsomes. Additionally, mitochondrial resting respiratory rates in brain, heart, and kidney tissues remain constant with age in diet restricted rats while they progressively rise in controls, with a corresponding increase in superoxide radical and hydrogen peroxide generation in the latter. This result supports the mitochondrial hypothesis in that dietary restriction successfully maintained the resting respiratory rate, showing that this method keeps the mitochondria functioning more efficiently over time.

Unmistakably, dietary restriction offers substantial health benefits due to its effect on modulating the production and metabolism of reactive oxygen species, or free radicals. Supplementing the restricted diet with powdered vacuum or freeze-dried vegetables and fruits that are naturally rich in antioxidants may provide additional health benefits. Epidemiological and clinical studies have revealed roles for antioxidants in the reduction of risk from cancer.

Introducing the possible connection between breast cancer and caloric restriction, Zhu et al. conducted a study of experimental mammary cancer in rats. The researchers found that caloric restriction correlated linearly with prolongation of latency to palpable carcinomas and a reduction in final incidence of mammary cancer. This experimental case may validate the potential use of caloric restriction to inhibit the conversion of precancerous cells to malignant cells. Caloric restriction also correlated with an increase in cortical steroid levels that explained 95% of the linear relationship between cancer and dietary restriction. Thus, dietary restriction may offer some chemoprotection by normalizing adrenal function.

DIETARY RESTRICTION AND HUMANS

A clinical study on the effects of dietary restriction was performed on four men and four women housed in Biosphere 2, a closed ecological space of 7 million cubic feet near Tucson, Arizona. As a result of poor food yields, the inhabitants of the biosphere consumed a low-calorie but nutrient dense diet for their two-year stay in the habitat. The men and women showed 18% and 10% weight loss, respectively. Further, the researchers measured blood lipids, glucose, insulin, glycosylated hemoglobin and renin in blood samples collected at several points during and after the two year period.

The nature of the largely vegetarian diet, with a high essential nutrient content per calorie, resembled that which retards aging, extends lifespan, and reduces age-related disease incidence in animals. Thus, Biosphere 2 offers an opportunity to study caloric restriction in humans under carefully monitored conditions and warrants deeper investigation.

The inhabitants of Biosphere 2 were all nonsmokers in good health. During the study each received three meals per day, and even though available food was less than expected, each received equal portions regardless of body size or gender. All of the inhabitants remained in excellent health throughout the two years, with the exception of minor ailments. The weight lost while in the biosphere was fully regained by six months after the end of the experiment and return to ad libitum diet.

Glucose, insulin, and glycosylated hemoglobin levels in the inhabitants all significantly decreased inside Biosphere 2.

It is important to note that Biosphere 2 crew members were calorie restricted but well supplied with all essential nutrients, so they were truly experiencing caloric restriction without malnutrition. Additionally, the crew members performed extensive physical and mental labor throughout the two years, which may have contributed to their good health.

This is the first study of caloric restriction in humans that can be compared in any meaningful way with corresponding observations in rodents and primates.
UNFRACTIONATED FRUITS AND VEGETABLES (WHOLE” FOODS) VS. SUPPLEMENTAL ISOLATES

In premenopausal women, a diet high in fruits and vegetables appears to be protective against breast cancer, especially if rich in specific carotenoids and vitamins.\(^\text{37}\) The protection is not bestowed by any single anticarcinogenic compound but appears to be a combined effect of numerous beneficial antioxidants and nutrients. Dietary antioxidants have been shown to protect against breast and several other cancer types. These antioxidants probably act by scavenging free radicals and quenching lipid peroxidation reactions, thus protecting cells from oxidative stress and damage. However, a large-scale study of 83,234 women examining the diet/cancer relationship found no association between cancer risk and dietary intake of vitamins C and E, a result supported by other studies.\(^\text{38-40}\) A recent study has suggested that vitamin C may be potentially carcinogenic by precipitating formation of lipid hydroperoxides that generate genotoxins,\(^\text{21}\) thus negating any radical scavenging activity. On the other hand, an inverse correlation between diet and risk of breast cancer has been found for carotenoids, vitamin A, lutein/zeaxanthin, and combined fruit and vegetable intake, with the strongest relationships in premenopausal women. These dietary factors had no apparent effect on risk in postmenopausal women.\(^\text{14}\)

While a high intake of fruits and vegetables correlates with decreased breast cancer risk, supplements of vitamins A, C, and E, and multivitamins were not associated with overall risk, supporting a whole food philosophy.\(^\text{14}\) Fruits and vegetables contain numerous tertiary, non-nutritive compounds including isoflavones, dithiolthiones, indoles, flavonoids, and phenols, all of which have proposed mechanisms of action and relative sites along the normal to abnormal cell transformation pathways that inhibit carcinogenesis and provide chemoprotection (Figure 1).

In addition, a diet high in fruits and vegetables reflects an overall healthier lifestyle and dietary pattern correlating with lower intake of saturated fat, less smoking, and more physical exercise.

XENOESTROGENS AND BREAST CANCER

Xenoestrogens are environmental compounds with estrogen-like activity that may cause hormone-related cancer in some individuals. Chlorinated pesticides, such as DDT and its metabolite DDE are examples of such compounds which have been linked to increased incidence of breast cancer,\(^\text{43-45}\) although other studies have found no link between breast cancer and serum levels of pesticides.\(^\text{46-51}\) For example, Wolff \textit{et al.}\(^\text{45}\) found that elevated levels of DDE and polychlorinated biphenyls (PCB) in the serum can result in a four-fold increased risk of breast cancer, while Krieger \textit{et al.}\(^\text{46}\) found no difference in serum levels of DDE and PCB in their prospective cohort study of 300 women. Interestingly, Krieger’s group did find that when the subjects were divided into racial subgroups, a risk was noticeable between black women and DDE levels. This association was further verified by Schildkraut \textit{et al.}\(^\text{52}\) who investigated the effect of body mass index (BMI—discussed briefly in the latter part of this paper) on plasma levels of DDE in black and white women. Results of multiple regression analyses indicated that black women did indeed have significantly higher plasma levels of DDE than white women. BMI was also found to be an independent predictor of DDE plasma levels in white and African-American women. Because the prevalence of obesity is higher among black women than white women, it may be possible that greater degrees of adiposity increase the body’s capacity to accumulate lipophilic contaminants such as DDT. Ethnic differences should certainly be considered in future studies examining the relationship between DDT, BMI and breast cancer risk. Until this occurs, and despite conflicting evidence regarding the links between xenoestrogens and breast cancer, it may be a good idea for women consuming large amounts of fruits and vegetables for breast cancer preven-

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**Figure 1.** The potential mechanisms and sites for the inhibition of carcinogenesis by protective tertiary compounds found within a whole food matrix.\(^\text{42}\)

<table>
<thead>
<tr>
<th>Pro-carcinogen</th>
<th>Key</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultimate carcinogen</td>
<td><strong>1</strong> Act as substrates/inhibitors of activating enzymes blocking the activation of the pro-carcinogen.</td>
</tr>
<tr>
<td>Normal Cell</td>
<td><strong>2</strong> Inactivate the ultimate carcinogenic species directly or by inducing deactivating enzymes, e.g. guanine reductase, catalase, superoxide dismutase, glutathione peroxide, UDP glucuronosyltransferases (UDPPTG), glutathione-S-transferase, etc.</td>
</tr>
<tr>
<td>Initiated Cell</td>
<td><strong>3</strong> Induce specific DNA repair enzymes.</td>
</tr>
<tr>
<td>DNA repair</td>
<td><strong>4</strong> Stimulate the process of apoptosis in initiated cells.</td>
</tr>
<tr>
<td>Transformed Cell</td>
<td><strong>5</strong> Inhibit cell division of regulate the induction and activity of specific hormones or membrane receptors for growth factors and nuclear gene expression systems.</td>
</tr>
</tbody>
</table>

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tion, to consider eating pesticide-free, certified organic produce whenever possible.

**BEEF AND PORK CONSUMPTION, OXIDATIVE DNA DAMAGE, AND BREAST CANCER**

NASA recognizes 5-hydroxymethyluracil as a biochemical marker of oxidative DNA damage that is positively associated with breast cancer risk (personal communication with NASA scientists at the Johnson Space Center Nutritional Biochemistry Laboratory). Djuric et al.\(^{53}\) measured serum levels of 5-hydroxymethyluracil in 21 healthy outpatient women with a first-degree relative with breast cancer. The women were assigned randomly to a low fat (N=9) or non-intervention (N=12) diet for 3-24 months. Diet data were obtained from 3-day food diaries. The researchers examined meat consumption by type (pork, beef, fish, or poultry) and by cooking temperature. They also examined intakes of either cooked or raw vegetables, respectively.

Intake of cooked vegetables and the sum of beef and pork intake explained the greatest variation (85\%) in DNA damage marker levels induced by diet. The preliminary results of this study suggest a positive association between levels of serum 5-hydroxymethyluracil and beef and pork intake, and a negative association with cooked vegetable intake.

Any model of breast cancer risk must take into account the complex interaction of covariables, including BMI, hormone levels, composition of fatty tissue, hormone receptor status, menopausal status, diet, and physical activity.

**COMPOSITION OF FATTY TISSUE**

In menopause, female steroid hormones are generated in fatty tissue by aromatase. The increased risk of breast cancer in overweight, menopausal women may have to do with elevated conversion of androgens to estrogens in fatty tissue. Estrogens then may stimulate mitotic activity in breast tissue leading to abnormal cell growth. Obesity also increases insulin levels and insulin resistance and decreases sex hormone binding globulin (SHBG) levels, all factors that influence risk.\(^{54}\) Insulin can increase proliferative activity of the breast epithelium. Thus, the mechanism may involve the interaction of diet with endogenous and exogenous estrogens, androgens, and glucose metabolic substances. If overweight is a major factor in postmenopausal breast cancer risk, weight reduction through caloric restriction, exercise, and nutritional supplementation may prove therapeutic. SHBG levels increase with weight loss, sometimes to levels higher than in healthy, non-obese, menopausal controls, offering significant protection from unopposed estrogen.\(^{54}\)

**GENETIC CONSIDERATIONS**

Genetic factors are an immutable risk factor and a history of familial breast cancer may modify associations between diet and breast cancer in premenopausal women.\(^{53}\) Nonetheless, lifestyle is not immutable and can be beneficially modified.\(^{54}\) Diets high in fruits and vegetables rely on an interaction of many variables for their chemoprotective effects and are probably beneficial regardless of genetic factors.

**BODY MASS INDEX (BMI) AND BREAST CANCER**

Body mass index (BMI) has been inversely correlated with premenopausal breast cancer risk but positively correlated with postmenopausal risk, a finding supported elsewhere (Table 4).\(^{54,15}\)

Maintaining a healthy body weight reduces the risk of many cancers in women, particularly breast and uterine.\(^{55}\) So-called “apple-shaped” women (those with adipose tissue around the waist and stomach) are 34\% more likely to develop breast cancer than “pear-shaped” women (those with adipose tissue around the hips and thighs) after menopause, especially if they have never taken hormone replacement therapy. Body shape does not appear to affect risk in premenopausal women, however.\(^{54}\) Women who gain significant weight in adulthood (after age 30) are more likely to develop uterine (endometrial) cancer and postmenopausal weight loss is suggested to decrease the risk.\(^{54,55}\)

Height, weight, BMI, and shape all seem to play a role in breast cancer development. For all age groups, height is found to correlate positively with breast cancer risk. Population studies in Europe have shown that height and age at the first term pregnancy describe almost 80\% of the total variation in breast cancer incidence. Conversely, BMI is negatively associated with risk in premenopausal women but positively associated in postmenopausal women. A possible confound to these results is that height is positively associated with osteoporosis in weight-bearing bones for which estrogen supplementation often is recommended in postmenopause. Thus, as height increases, so does the tendency for estrogen supplementation, which increases the risk for estrogen-sensitive breast cancer.\(^{54}\)

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Premenopausal OR</th>
<th>Postmenopausal OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 21.7</td>
<td>1 (^{1})</td>
<td>1 (^{1})</td>
</tr>
<tr>
<td>21.7-23.7</td>
<td>0.93 (0.7-1.2)</td>
<td>1.00 (0.8-1.3)</td>
</tr>
<tr>
<td>23.8-25.7</td>
<td>0.85 (0.6-1.1)</td>
<td>1.07 (0.8-1.4)</td>
</tr>
<tr>
<td>25.8-28.8</td>
<td>0.89 (0.6-1.2)</td>
<td>1.21 (1.0-1.5)</td>
</tr>
<tr>
<td>≥ 28.9</td>
<td>0.67 (0.5-0.9)</td>
<td>1.39 (1.1-1.8)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^{1}\) Reference category

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Djuric et al., 53 mea... 5-hydroxymethyluracil as a biochemical marker of oxidative DNA damage that is positively associated with breast cancer risk (personal communication with NASA scientists at the Johnson Space Center Nutritional Biochemistry Laboratory).
Nutrition during periods of rapid growth (childhood and puberty) seems to be an important factor for final height. As a result, height may only be a proxy variable of nutritional status during the years of growth with the latter being the true risk factor for breast cancer. Indeed, energy intake in childhood has been associated with the development of cancers later in life. This would also support caloric restriction research suggesting that reduced caloric intake lengthens the time to onset of some disease states.

The age at menarche is related to childhood nutritional status and an earlier age corresponds to increased nutrition and increased cancer risk. Further, a growth spurt in the mammary glands during adolescence is thought to be responsible for the final number of mammary cells that are vulnerable to carcinogenic transformation. An increase in weight during this period is associated with anovulatory cycles that limit this growth spurt. Thus, weight gain during adolescence may reduce premenopausal breast cancer risk by reducing the number of cancer-vulnerable cells.

Contrary to much public opinion, dietary fat does not appear to be a significant risk factor linking weight and cancer risk. In fact, monounsaturated fats may be cancer protective.

Regular exercise is very important also. It may reduce a woman’s risk of cancer by as much as 30%. In fact, BMI may be a proxy variable for physical activity because exercise correlates with a lower BMI and with lower risk.

The specific impact that a change in diet may have on a woman’s risk of breast cancer, depending upon the presence of a gene mutation, her BMI, her ethnic background, serum levels of SHBG and other metabolic-endocrine factors is not clear at this time. Further research needs to be done prior to proposing an explanatory model of the interconnections between such confounding variables.

**PHYTOESTROGENS**

In vitro, the soy phytoestrogens genistein, daidzein, biochanin A, and coumestrol, as well as the extracts of several herbs, were tested for their effects on the growth of breast cancer cell lines T-47D and MCF-7. The isoflavones significantly inhibited the growth of both cell types in the range of 10-100 mM, but no effect was observed below 1 mM. Over three days coumestrol was the most inhibitory, followed by genistein, daidzein, and biochanin A (Figure 2). The result roughly equates to the putative estrogenic activity of the isoflavones. Biochanin A, the weakest, is methoxylated, which abolishes its estrogenicity. Hops, black cohosh root, dong quai root, and vitex berry all inhibited growth significantly at full strength, while ginseng was ineffective.

Zava et al. reported on the content and bioactivity of plant phytoestrogens and progestins in various foods, plants, and spices before and after human consumption.

- 150 plants were extracted and tested for their ability to compete for estradiol and progesterone binding to intracellular estradiol (ER) and progesterone (PR) receptors in intact human breast cancer cells. The seven highest ER-binding plants, foods and spices were: soy, licorice, red clover, thyme, turmeric, hops, and verbena. The six highest PR-binding plants, foods and spices were: oregano, verbena, turmeric, thyme, red clover, and damiana (Table 5). The foods, plants, and spices that showed high levels of phytoestrogens and phytoprogestins were further tested for bioactivity in regulating the growth of ER positive and ER negative breast cancer cell lines and in inducing or inhibiting the synthesis of alkaline phosphatase, an end product of progesterone action, in PR positive cells. ER-binding plant extracts demonstrated agonist activity, acting in a way similar to estradiol. Conversely, PR-binding extracts were neutral or acted as antagonists of progesterone, in general.

In ER positive T47D cells, licorice, red clover, yucca, hops, and motherwort stimulated significantly higher growth than control, with yucca and red clover found to be nearly equipotent with estradiol at the concentrations tested. Most ER-binding extracts were neutral on growth of ER negative MDA468 cells, but interestingly, mandrake, bloodroot, mistletoe, and juniper actually markedly inhibited cell growth in ER negative MDA468 cells (Figures 3 and 4), suggesting these foods and plants are chemoprotective via an estrogen receptor-independent mechanism.

Dilute extracts of high PR-binding plants were incubated with T47D cells and monitored for alkaline phosphatase activity. Controls were progesterone and progesterone+RU486, a potent antiprogestin. None of the extracts significantly elevated alkaline phosphatase, categorizing them as either neutral or antagonists of progesterone.
Damiana, dong quai, yucca, and mistletoe failed to competitively inhibit the induction of alkaline phosphatase by exogenous progesterone, categorizing them as neutral. Conversely, red clover, licorice, goldenseal, pennyroyal, and nutmeg either completely or partially blocked alkaline phosphatase induction by progesterone, categorizing them as putative antiprogestins.

### Table 5. Herbs and spices containing ER-and PR-binding components

<table>
<thead>
<tr>
<th>ER-binding*</th>
<th>PR-binding**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy milk</td>
<td>8/200cc</td>
</tr>
<tr>
<td>Licorice</td>
<td>4/2g</td>
</tr>
<tr>
<td>Red clover</td>
<td>3</td>
</tr>
<tr>
<td>Mandrake</td>
<td>3</td>
</tr>
<tr>
<td>Bloodroot</td>
<td>2</td>
</tr>
<tr>
<td>Thyme</td>
<td>2</td>
</tr>
<tr>
<td>Yucca</td>
<td>0.5</td>
</tr>
<tr>
<td>Turmeric</td>
<td>0.5</td>
</tr>
<tr>
<td>Hops</td>
<td>0.5</td>
</tr>
<tr>
<td>Verbena</td>
<td>0.5</td>
</tr>
<tr>
<td>Yellow</td>
<td>0.5</td>
</tr>
<tr>
<td>Sheep sorrel</td>
<td>0.5</td>
</tr>
<tr>
<td>Ocotillo</td>
<td>-</td>
</tr>
<tr>
<td>Oregano</td>
<td>-</td>
</tr>
<tr>
<td>Damiana</td>
<td>-</td>
</tr>
<tr>
<td>Pennyroyal</td>
<td>-</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>-</td>
</tr>
<tr>
<td>Calamus root</td>
<td>-</td>
</tr>
<tr>
<td>Goldenseal</td>
<td>-</td>
</tr>
<tr>
<td>Mistletoe</td>
<td>-</td>
</tr>
<tr>
<td>Cumin</td>
<td>-</td>
</tr>
<tr>
<td>Fennel</td>
<td>-</td>
</tr>
<tr>
<td>Chamomile</td>
<td>-</td>
</tr>
<tr>
<td>Cloves</td>
<td>-</td>
</tr>
</tbody>
</table>

* (mcg estradiol equivalents/200cc or 2g dry herb)
** (mcg progesterone equivalents/2g dry herb)

THE 2-HYDROXYESTRONE: 16A-HYDROXYESTRONE (2:16) RATIO AND BRASSICA VEGETABLES

16α-hydroxyestrone (16HE) is known to increase and to promote breast cell proliferation in vitro by binding covalently to estrogen receptors on breast cells, and to promote mammary tumors in mice.62,63 Another metabolite of estro-
**Figure 5** Menopause, HRT Contraindications, and Associated Dietary Considerations

- Cessation of ovarian function caused by natural physiologic menopause including associated phases:
  - Premenopause Begins after age 40 in women
  - Perimenopause Transitional phase
  - Menopause Last spontaneous menstrual bleeding

- Associated Signs and Symptoms:
  - Hot flashes
  - Profuse perspiration
  - Headache
  - Vertigo
  - Heart palpitation
  - Ringing in ears
  - Nervousness/irritability
  - Sleep disturbances
  - Depressive moods
  - Joint pain
  - Loss of concentration

- Surgical removal of the ovaries and uterus
- Radiation of the ovaries

- Patient has history of current diagnosis or presenting signs & symptoms of:
  - Cancer
  - Endometriosis
  - Fibrocystic Breast Disease
  - Uterine Fibroids
  - Coronary Artery Disease
  - Angina
  - Unexplained uterine bleeding
  - Liver & gallbladder disease
  - Pancreatitis

- Menopause
- Patient refuses synthetic HRT
- Synthetic hormone replacement therapy (HRT) contraindicated

Consider diet modification guidelines suggested in research studies to reduce symptoms of menopause and prevent risk of endometrial and breast cancer:

- Consume vegetables of the Brassica genus (e.g., broccoli, kale and Brussels sprouts) to potentially shift estrogen metabolism and increase the 2-hydroxyestrone:16a-hydroxyestrone (2:16) ratio.\(^{57-71}\)
- Consider a calorie restricted diet.\(^{18-36}\)
- Consume whole foods high in antioxidants rather than isolated antioxidant vitamins.\(^{14,29,37,74-79}\)
- Limit intake of breads and cereal dishes, saturated fat and alcohol. Replace with high quality seed oils, fish and raw vegetables.\(^{15}\)
- Limit intake of beef and pork.\(^{83}\)
- Eliminate intake of well-done meat, particularly in women with the rapid/intermediate NAT2 genotype to reduce breast cancer risk in postmenopausal women.\(^{72}\)
- Consider incorporating Clmcifuga racemosa and other estrogenic plants into protocol to reduce symptoms of menopause.\(^{80,81}\)
- Increase intake of phytoestrogens (for example legumes such as red clover, peas, beans, lentils and other podded plants) and consume unFractionation fruits and vegetables (preferably organic)\(^{43-52}\) rather than isolated antioxidant supplements to impart an antiestrogenic effect, inhibit tyrosine-specific protein kinases and angiogenesis, provide an antioxidative effect, and induce sex hormone-binding globulin.\(^{59}\)
gen, 2-hydroxyestrone (2HE) is not known to be carcino-
genic. It appears to have anti-estrogenic properties in breast
tissue and may inhibit angiogenesis. In humans, these
two metabolites are products of alternate and irreversible
metabolic pathways such that the ratio of E2:E16 may be a
putative endocrine biomarker for estrogen-related breast
cancer risk in humans. Any mechanism that shifts estro-
gen metabolism toward 2HE hypothetically would lower
breast cancer risk from 16HE.

Interestingly, brassica vegetable consumption has been
shown to significantly increase the 2HE:16HE ratio in
healthy postmenopausal women consuming brassicas
approaching levels equivalent to 70 mg/day of glucosino-
lates. The health-promoting indole and isothiocyanate
breakdown products of glucosinolates are known to stimu-
late Phase I and Phase II detoxification enzymes. Phase
I cytochrome P-450 enzymes hydroxylate estrogen to 2HE,
depleting the pool available for conversion to 16HE, consistent with reducing breast cancer risk.

N-ACETYLTRANSFERASES (NAT)

N-acetyltransferases (NAT) are major enzymes of
breast tissue that activate aromatic and heterocyclic amines,
such as those found in cigarette smoke and well-cooked red
meat. In addition to explaining the generally increased
cancer risk from smoking, these researchers also found that
certain polymorphisms, or genetic variations, of NAT alle-
les found in humans are significantly more highly correlat-
ed with breast cancer risk due to smoking and red meat con-
sumption than others. These alleles code for the
“rapid/intermediate acetylator phenotype” in which hetero-
cyclic amines are more quickly activated, increasing the
risk of toxic DNA damage leading to cancer. Women with the
rapid/intermediate acetylator phenotype may be at sig-
nificantly higher risk for breast cancer when they smoke
and consume meat cooked at high temperatures.

OVERVIEW

The research studies cited in this review article reveal
that hormone replacement therapy (HRT) increases a
woman’s risk of breast cancer. Figure 5 offers summary of
the dietary factors that may reduce breast cancer risk in
women and aid in menopausal hormonal balance. While
HRT increases breast cancer risk, caloric restriction and the
use of phytoestrogenic and phytoprogesterenic foods may
reduce the risk. Interestingly, in most cases, a diet high in
unfractionated fruits and vegetables is also protective
against breast cancer although the protection is not
bestowed by any single anti-carcinogenic compound found
within fruits and vegetables. While BMI, insulin levels,
incidence of insulin resistance, sex hormone binding glob-
ulin (SHBG) levels and exposure to xenoestrogens all seem
to be connected to breast cancer risk, more research is war-
ranted to further evaluate the relationship between these
variables, particularly in varying ethnic groups, as they may
be potentially important modifying factors for breast cancer
risk. Addressing biochemical individuality as much as is
possible in future diet and herb-related breast cancer stud-
ies will undoubtedly provide us with a clearer assessment
of the role that foods, diets and herbs play in the prevention
and/or treatment of breast cancer.

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Pilot Study: An Emulsified Fish Oil Supplement Significantly Improved C-Reactive Protein, Hemoglobin, Albumin and Urine Output in Chronic Hemodialysis Volunteers

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who were undergoing treatment with chronic intermittent maintenance hemodialysis at a Minnesota clinic, and at various Fresenius clinics in Houston, Texas.

Results: Serum albumin, hemoglobin, and urine volume increased, while CRP measurably decreased. Concomitantly, the amount of erythropoietin needed per treatment to maintain adequate non-anemic Hgb levels fell (mean Hgb [Erythropoietin]: initial Hgb 12.05+0.83 [Epo. 6346+2556.65] vs final: Hgb 12.25+0.64 [Epo. 5336+2399.27] ) (P < .05).

Conclusion: A highly bioavailable emulsified omega-3 fatty acid dietary supplement positively impacted hemoglobin, serum albumin, urine output, and CRP values. Volunteers, completing additional subjective tests (urine foaming and joint pain), indicated a substantial decrease in both. Considering the impact this highly bioavailable dietary supplement had on improving hematological values and in reducing the total required dose of erythropoietin needed per treatment to maintain adequate hemoglobin levels, a major study is warranted.

Key Words: Emulsified fish oil, hemoglobin, erythropoietin, C-reactive protein, nutritional supplement, Coromega™

INTRODUCTION

Both acute and subclinical inflammatory states can be characterized by an excessive release of a variety of inflammatory factors. This antagonistic molecular cascade can, in turn, exert a negative influence on every physiological system, and has been implicated as a forerunner of atherosclerotic plaques in both the normal and ESRD populations. Elevated C-reactive protein (CRP), an easily identifiable

ABSTRACT

Objective: Elevated C-reactive protein (CRP) levels, in conjunction with diminished albumin and hemoglobin (Hgb) values, are associated with increased morbidity and mortality in patients with end stage renal disease (ESRD). Evidence suggests that a number of factors, including dialysis, alone or in combination with erythropoietin administration, can cause CRP values to rise. Omega-3 fatty acids have been shown to exhibit significant anti-inflammatory/antioxidant activity affecting a wide variety of disorders. A highly bioavailable omega-3 dietary fatty acids supplement was tested for its ability to suppress CRP, and positively impact serum albumin and hemoglobin values, as well as urine output and pain.

Methods: ESRD volunteers took 3 packets of Coromega™ each containing 650 mg of emulsified fish oil (350 mg of EPA, and 230 mg of DHA) for a total of 1,950 mg of emulsified omega-3 fatty acids per day for a period of 8 consecutive weeks. Serum albumin, CRP, hemoglobin and erythropoietin usage was measured at specific intervals during the test period. Volunteers were asked to measure urine output and rate pain on specific days.

This pilot study was performed with ESRD volunteers...
non-specific inflammatory marker, is an acute phase protein, produced in response to an inflammatory event (cytokine activation) regardless of its cause.1

Many hemodialysis patients have high levels of CRP. Repeated exposure to artificial membranes (dialysis membrane, synthetic tubing, and containers),2-3 erythropoietin administration, as well as idiopathic causes native to ESRD conditions, have all been implicated as having the potential to evoke an inflammatory response. In addition to atherosclerotic plaques,4 an elevation in CRP has been associated with an increase in cardiovascular disease,5 decrease in serum albumin synthesis,6,7 and an increase in erythropoietin resistance with ensuing anemia in ESRD. Elevated CRP levels, in conjunction with diminished albumin and hemoglobin values, are associated with increased morbidity in the ESRD patient maintained on chronic hemodialysis, and can be viewed as a strong predictor of poor survival.

Significant anti-inflammatory properties have been associated with fish oil (omega-3 fatty acids) supplements. Immune-activated T cells and cytokines are found throughout the circulatory system as well as at sites of injury. Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) will generate reactive oxygen intermediaries, which may contribute to free radical cell and tissue damage. These reactive intermediaries can, in turn, attack cell membrane polyunsaturated fatty acids, resulting in derivatives that subsequently attract inflammatory cells. In their unoxidized form, omega-3 fatty acids may inhibit circulatory inflammation by decreasing the precursors, which, in themselves, may lead to free radical mediated events.8,9,10,11,12

Omega-3 fatty acids have been shown to inhibit the production of eicosanoids (proinflammatory compounds). Arachidonic acid (an omega-6 fatty acid) is metabolized into one such proinflammatory eicosanoid. Conversely, omega-3 fatty acids can displace arachidonic acid from the cell membrane reducing membrane bound proinflammatory compounds. Omega-3 also competes with arachidonic acid for cyclooxygenase and lipoxygenase enzymes, shifting eicosanoid production to the non-inflammatory series-3 prostaglandins and series-5 leukotrienes. This specific competitive activity has been hypothesized to directly suppress immunological and/or inflammatory mediators.

A number of other statistically significant, physiologically positive events have been associated with omega-3 fatty acid supplementation when given at doses between 1.8 -18 grams per day. Those specifically noted include: mild antithrombotic effects,13 a mild glomerular protective effect (by limiting production of cytokines and eicosanoids evoked by immunologic renal injurious events),14 a mild antihypertensive effect in persons with confirmed hypertension,15,16 anti-inflammatory benefits associated with emphysema,17 asthma,18 rheumatoid arthritis,19,20 and increased hemoglobin in ESRD volunteers.21 In the case of hemoglobin production, two explanations have been postulated: (1) hemoglobin production increases because inflammatory factors (as well as free oxygen radicals) are reduced, lessening the negative impact on the system (body) as a whole, and (2) a protective effect is being exerted by omega-3 fatty acids on the red cells, preventing early cell death (the average lifespan of a red blood cell is 120 days in the normal individual vs 40 for the dialysis patient). While the mechanism for these numerous positive observations, seen across an entire spectrum of disorders, is not fully understood, a hypothesis, focusing on cell and tissue oxidative and inflammatory processes as a basis for the initiation of all organ (and aging related) deleterious events, would seem a logical place to begin.

The “effective dose” of fish oil, necessary to attain the results observed in each study, has varied greatly from study to study, for both the same, and dissimilar, disease conditions. Possible explanations for this variation may have to do with the preoxidized state, and/or bioavailability of the fish oil preparation being used. Fish oil can oxidize when exposed to air, both during processing and storage. The degree to which it oxidizes varies with the length of time exposed and the prevailing temperature. Significant antioxidative activity has been associated with fish oil. If its affinity for oxidative free radicals is, at least in part, the mechanism by which it exerts its biological affect, the effectiveness of emulsified fish oil could be significantly reduced after oxidation. The bioavailability of any one specific preparation would also be a key factor in the amount needed to achieve a desired affect.

The fish oil preparation Coromega™ (manufactured by the European Reference Botanical Laboratories) was selected for use in this pilot study because of its reported high bioavailability and hermetically sealed packaging. The bioavailability of 4 grams of this emulsified and flavored fish oil product was compared to a standard, commercially available, encapsulated fish oil in a feeding trial using non-dialyzing men. Findings indicated that the percent of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids assimilated into the bloodstream from the emulsified product reached approximately 30% at 8 hours, compared to approximately 10% for the traditional encapsulated fish oil group for the same time period. Incorporation percentage remained uniformly higher at 48 hours for the emulsified fish oil, compared to the encapsulated version (>20% vs >10%).22

The objectives of this pilot study were to determine if this highly bioavailable fish oil preparation was capable of causing a statistically significant reduction in the amount of erythropoietin needed to maintain a non-anemic state, a reduction in CRP values, while concomitantly stimulating increased production of serum hemoglobin, and albumin. Because this was a pilot study with strictly limited funded resources, the authors sought to maximize the observations possible in this study by enlisting the volunteers aid in
observing and recording data for the following subjective aspects of this study: (I) urine foaming, (II) increase (or decrease) in urine production, and (III) reduction (or increase) in joint pain. Subjective parameters involved at-home recording of urine protein spillage by observing urine “foam forming” capacity, urine volume, and rating joint pain (on a scale of 0 to 10) for 60 consecutive days.

SUBJECTS AND METHODS

Subjects: Subjects were fully informed of the nature of the pilot study in which they were being asked to participate, and each volunteer provided written consent. The pilot study protocol conformed to the policies for research on human subjects, and the Medical Director’s approval was obtained. Additionally, Medical Director oversight continued throughout the duration of the study. Twenty adult hemodialysis ESRD volunteers (mean 55.9 years) of either gender, having both of their original kidneys, and who had been maintained on chronic hemodialysis for at least 4 months (labs drawn at regular intervals and data collected), were recruited into this pilot study where the volunteers acted as their own controls. Approximately 30% of all volunteers had ESRD secondary to unspecified hypertensive renal disease, ESRD diabetic-related 30%, Lupus-related 12%, ischemic 6%, other 22%. An effort was made to recruit individuals with high initial CRP (>10 mg/L) values and/or relatively large erythropoietin unit needs (>10,000 units). Qualitative analysis was used to measure CRP; therefore values less than 6.9 were undetected. For statistical analysis, those patients with a CRP < 6.9, were recorded as 6.8. This method allowed for the most conservative treatment of the data set. With the exception of one volunteer, who was admitted to the pilot study because of an exceptionally large erythropoietin unit need, none of the other volunteers were taking a prophylactic oral anticoagulant (warfarin sodium).

Intervention: Each participant took 3 packets of Coromega™, each containing 650 mg of emulsified fish oil (350 mg of EPA and 230 mg of DHA) or 1,950 mg of emulsified omega-3 fatty acids per day, for a period of 8 consecutive weeks. Serum albumin, CRP, hemoglobin and erythropoietin usage was measured prior to and at specific intervals during the test period. In addition to omega-3 fatty acids, each packet of the product also contained: 8 mg of cholesterol (from 1:80 of a pasteurized egg yolk), 25 mg of vitamin C, 3 IU of vitamin E, 50 micrograms of folic acid, and 5 mg of stevia leaf extract.

Interested volunteers were asked to measure urine output on specific days, and count free floating foam (greater than 3 inches in diameter), after urinating in a toilet, as “+” or less than 3” as “−”. Individuals so inclined kept a “pain” diary scoring their pain on a scale of 0 to 10 each day (where 0 = no pain, and 10 = severe and intolerable pain).

Erythropoietin Administration: The amount of erythropoietin administered per treatment is listed in Table 3 (under “EPO”). The number of erythropoietin units administered to each volunteer during the pilot study was closely monitored and was gradually decreased to keep pace with any rises observed in the volunteer’s hemoglobin level. The target hemoglobin level was at or above 12.1 mg/dL. Erythropoietin was “held” only when the volunteer’s hemoglobin level rose above 13 mg/dL (in accordance with the dialyzing center’s policy for erythropoietin administration).

Data: Predialysis data were obtained for all parameters: CRP and albumin (initial and at week 8), hemoglobin and erythropoietin units administered (initial and at two week intervals), urine foaming and volume (initial and at weekly intervals), and pain (scored daily).

Analysis: Only data from participants who completed all 8 weeks, taking 3 packets per day, were used. Percentage of decrease and increase for all values was calculated at the conclusion of the pilot study, and the results expressed as an average reduction or increase for each test parameter. All data were further analyzed using t-test: paired two sample for means.

Due to the data set’s small size and large variance, change in C-reactive protein values (Table 1) was not statistically significant even though reductions in CRP for many of the volunteers were observed. An overall change of 12.6 % was noted among the means of CRP (CRPmean initial of 19.09 mg/L vs CRPmean final of 16.67 mg/L). However, with large variances of 234.9 and 218.9 respectively, no statistical significance could be shown. This does not discount that 7 of 14 patients showed a marked decrease in CRP after treatment.

Albumins (Table 2), approached statistical significance in change of the means (Albmean initial of 3.86 g/dl vs Albmean final of 3.99 g/dl) (P = 0.056). Change of the means for hemoglobin (Hgb) and erythropoietin (Epo) were compared from week 0 to week 8. As designed, there was no significant change in Hgb from the initial lab draw to the final lab draw (Hgbmean initial of 12.05 g/dl compared to Hgbmean final of 12.25 g/dl). However, Epo use was significantly reduced (16%) from a mean dose of 6346 units to 5336 units three times per week (P .05).

RESULTS

Of the 20 initial recruits, data was obtained for 14 (70%) volunteers for CRP, 18 (90%) for albumin, and 11 (55%) for hemoglobin and erythropoietin. Missed samplings accounted for the discrepancy in the number of participants sampled for each parameter. Volunteers compliance in subjective testing was substantially less than that of
the objective. Five volunteers (25%) elected to participate in the “foaming” study, three (15%) in the urine volume study, and two (10%) in the pain study.

As shown in Table 1, CRP was reduced 5-89%. The mean initial CRP was 19.09±8.85 mg/L (SD 15.33) vs mean final CRP of 16.67±8.54 mg/L (SD 14.80).

As shown in Table 2, albumin values increased 2-19%. The mean initial albumin was 3.86±0.20 g/dl (SD 0.40) vs a mean final albumin of 3.99±0.14 g/dl (SD 0.29).

As shown in Table 3, by the end of the study hemoglobin increased 2-25%. The initial mean Hgb was 12.05±0.83 g/dl (SD 1.24) vs the final mean Hgb of 12.25±0.64 g/dl (SD 0.96). The amount of erythropoietin needed to combat anemia decreased. Initial mean erythropoietin use was 6346±2557 units (SD 3805.62) vs final mean erythropoietin use of 5336±2399 units (SD 3571.35).

At the beginning of the pilot study, urine foaming was uniformly noted by each participant. Four of the five participants reported a cessation in foaming beginning with the first week and continuing through the eighth. During the study, none of the participants reported that they had consumed an excess of fluid during any particular week. Each of the three participants measuring their urine volume reported increases in urine output. While these increases occurred almost every week, no conclusions from the numbers generated could be drawn because they never reached a statistically significant level.

Both volunteers scoring daily pain indicated substantial reductions in pain intensity. While pain reduction was noted during the first week of the study, it was reported to be “very pronounced” after day 20.

DISCUSSION

Volunteers responded positively to the emulsified fish oil preparation used in this pilot study. Comments concerning “fishy burps”, “fishy after taste”, or “fishy indigestion” were absent with this preparation.

As observed, most of the participant’s test parameters were positively influenced. There appeared to be no consistent pattern with respect to the few individuals for whom one or more “negative” values were generated. For example, volunteer #109 returned the highest CRP value and the largest decrease in hemoglobin, yet experienced a rise in albumin, or increase in urine volume, and a significant decrease in pain scores. Volunteer #114 attained the second highest hemoglobin increase of the group, but scored the second largest increase in CRP value. Volunteer #111 scored the highest increase in urine output and second largest decrease in pain scores, yet experienced a slight decrease in hemoglobin value. No bleeding episodes or periods of illness were noted for any volunteer, and we are unable to give a plausible explanation for the sharp increase in CRP, or decrease in hemoglobin (for volunteer 109 and 111, respectively) noted on the final test.

Of special interest was the highly significant impact (P < 0.05) the emulsified fish oil preparation had on the number of units of erythropoietin (6346±2557 reduced to 5336±2399) needed to maintain hemoglobin at, or above, 12.1 mg/dL (a recognized non-anemic state). There was no significant change in mean hemoglobin between the first and eighth week of the pilot study (12.05±0.83 vs 12.25±0.64). The reduced need for erythropoietin to maintain adequate hemoglobin levels during emulsified fish oil supplementation was a paramount finding in this pilot study.

The reduced erythropoietin usage observed in this study represents an estimated dollar saving to the patients (and the healthcare system) of between $200,000-$300,000 per 100 patients/year (based on $100-$150/10,000 units cost). While the small sample size of this pilot study limits the absolute conclusiveness of these findings, the impact of their potential cannot be ignored. If the same results can be realized in a larger trial, one could easily extrapolate the expected increase in patient quality of life health benefits and substantial decrease in cost of erythropoietin used. Given the significance of the findings, in the absence of any adverse events, a larger trial is warranted.

ACKNOWLEDGEMENTS

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A special thanks to Frank Morley and Barbara Apps of the European Reference Botanical Laboratories, Inc. for their support and encouragement throughout the study.
### Table 1. C-Reactive Protein Values (mg/L)

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<th>Final (8 Weeks)</th>
<th>% Reduction</th>
<th>Increase</th>
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NS (-) = Not statistically significant

### Table 2. Albumin (g/dL) Values

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</tr>
<tr>
<td>#115</td>
<td>3.9</td>
<td>3.7</td>
<td>-5%</td>
<td></td>
</tr>
<tr>
<td>#116</td>
<td>4.0</td>
<td>4.3</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>#117</td>
<td>3.6</td>
<td>3.8</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>#118</td>
<td>3.5</td>
<td>3.8</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.86+0.20</td>
<td>3.99+0.14</td>
<td>Change: (3.6%, p = 0.056)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3. Hemoglobin (Hgb g/dL) vs. Erythropoietin (EPO) Administered (U/dialysis session)

<table>
<thead>
<tr>
<th>#</th>
<th>Initial Hgb (EPO)</th>
<th>Week 2 Hgb (EPO)</th>
<th>Week 4 Hgb (EPO)</th>
<th>% Increase/Decrease in Hgb</th>
<th>Week 6 Hgb (EPO)</th>
<th>Week 8 Hgb (EPO)</th>
<th>% Increase/Decrease from initial value</th>
</tr>
</thead>
<tbody>
<tr>
<td>#101</td>
<td>11.9 (1,800)</td>
<td>12.3 (1,800)</td>
<td>12.5 (1,500)</td>
<td>5%</td>
<td>12.7 (1,500)</td>
<td>12.4 (1,500)</td>
<td>4% [-300 U]</td>
</tr>
<tr>
<td>#109</td>
<td>14.5 (2,000)</td>
<td>13.1 (1,500)</td>
<td>12.7 (1,500)</td>
<td>(-12%)</td>
<td>13.2 (1,000)</td>
<td>11.9 (1,000)</td>
<td>[-18%] [-1,000 U]</td>
</tr>
<tr>
<td>#110</td>
<td>12.5 (4,500)</td>
<td>11.8 (4,500)</td>
<td>12.8 (4,500)</td>
<td>2%</td>
<td>12.4 (4,000)</td>
<td>12.8 (4,000)</td>
<td>2% [-500 U]</td>
</tr>
<tr>
<td>#111</td>
<td>12.3 (5,500)</td>
<td>11.5 (5,500)</td>
<td>11.4 (5,000)</td>
<td>(-7%)</td>
<td>11.2 (5,000)</td>
<td>12.1 (5,500)</td>
<td>[-8%] 0 U</td>
</tr>
<tr>
<td>#112</td>
<td>12.2 (7,500)</td>
<td>12.0 (7,500)</td>
<td>13.6 (5,500)</td>
<td>10%</td>
<td>13.0 (5,500)</td>
<td>13.4 (5,500)</td>
<td>10% [-2,000 U]</td>
</tr>
<tr>
<td>#113</td>
<td>12.1 (500)</td>
<td>12.2 (500)</td>
<td>12.4 (500)</td>
<td>2%</td>
<td>12.0 (200)</td>
<td>12.5 (200)</td>
<td>3% [-300 U]</td>
</tr>
<tr>
<td>#114</td>
<td>11.4 (10,000)</td>
<td>12.6 (9,500)</td>
<td>13.9 (hold)</td>
<td>18%</td>
<td>14.7 (hold)</td>
<td>13.3 (5,000)</td>
<td>14% [-5,000 U]</td>
</tr>
<tr>
<td>#115</td>
<td>12.7 (6,500)</td>
<td>12.5 (6,000)</td>
<td>12.2 (6,000)</td>
<td>(-4%)</td>
<td>12.6 (6,000)</td>
<td>12.7 (6,000)</td>
<td>0% [-500 U]</td>
</tr>
<tr>
<td>#116</td>
<td>9.2 (11,000)</td>
<td>9.9 (11,000)</td>
<td>10.7 (11,000)</td>
<td>14%</td>
<td>11.3 (11,000)</td>
<td>12.3 (10,000)</td>
<td>25% [-1,000 U]</td>
</tr>
<tr>
<td>#117</td>
<td>11.8 (10,000)</td>
<td>11.5 (10,000)</td>
<td>11.6 (10,000)</td>
<td>(-2%)</td>
<td>11.3 (10,000)</td>
<td>11.3 (10,000)</td>
<td>[-4%] 0 U</td>
</tr>
<tr>
<td>#118</td>
<td>11.9 (10,500)</td>
<td>11.7 (10,500)</td>
<td>12.2 (9,000)</td>
<td>3%</td>
<td>12.0 (9,000)</td>
<td>10.0 (10,000)</td>
<td>[-16%] [-500 U]</td>
</tr>
</tbody>
</table>

**Mean Hgb (EPO):**

**Initial:** 12.05±0.83 (6346±2556.65)  
**Final:** 12.25±0.64 (5336±2399.27)  
**Change:** NS (-16%, p<0.05)

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### REFERENCES


